2023-06

Effect of Teff Flour Particle Size and Kneading Time on Dough Rheological Property and Injera Quality

Berhanu, Regassa Jima

BAHIR DAR UNIVERSITY

BAHIR DAR INSTITUTE OF TECHNOLOGY

SCHOOL OF GRADUATE STUDIES

FACULTY OF CHEMICAL AND FOOD ENGINEERING

MASTER OF SCIENCE PROGRAM IN FOOD TECHNOLOGY

MSc Thesis on:

Effect of Teff Flour Particle Size and Kneading Time on Dough Rheological Property and Injera Quality

By

Berhanu Regassa Jima

June, 2023

Bahir Dar, Ethiopia

BAHIR DAR UNIVERSITY

BAHIR DAR INSTITUTE OF TECHNOLOGY

FACULTY OF CHEMICAL AND FOOD ENGINEERING

Effect of Teff Flour Particle Size and Kneading Time on Dough Rheological Property and Injera Quality

By

Berhanu Regassa Jima

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Food Technology

Advisor: Agimassie Agazie Abera (Asst. Professor)

June, 2023

Bahir Dar, Ethiopia

BAHIR DAR UNIVERSITY

BAHIR DAR INSTITUTE OF TECHNOLOGY

SCHOOL OF GRADUATE STUDIES

FACULTY OF CHEMICAL AND FOOD ENGINEERING

Approval of Thesis for Defense

I hereby certify that I have supervised, read, and evaluated this work titled " Effect of Teff Flour Particle Size and Kneading Time on Dough Rheological Property and Injera Quality" prepared by Berhanu Regassa under my guidance. I recommend that the thesis be submitted for oral defense.

Agimassie Agazie Abera (Asst. Professor)	[signature]	18/07/2023
Advisor's name	Signature	Date

BAHIR DAR UNIVERSITY

BAHIR DAR INSTITUTE OF TECHNOLOGY

SCHOOL OF GRADUATE STUDIES

FACULTY OF CHEMICAL AND FOOD ENGINEERING

Approval of Thesis for Defense

I hereby confirm that the changes required by the examiners have been carried out and incorporated in the final thesis.

Name of Student **Berhanu Regassa** Signature ____ Date 14/07/2023

As members of the board of examiners, we examined this thesis entitled "Effect of Teff Flour Particle Size and Kneading Time on Dough Rheological Property and Injera Quality" By Berhanu Regassa Jima. We hereby certify that the thesis is accepted for fulfilling the requirements for the award of the degree of Masters of Science in "Food Technology".

Board of Examiners

	Signature	Date
Name of Advisor		
Mr. Agimassie Agazie		18/07/2023
Name of External examiner		
Henock Woldemichael (PhD)		15 July 2023
Name of Internal Examiner		
Metadel Kasahun (PhD)		18/07/2023
Name of Chairperson		
Biresaw Demelash (PhD)		18/07/2023
Name of Chair Holder		
Biresaw Demelash (PhD)		18/07/2023
Name of Faculty Dean		
Metadel Kasahun (PhD)		

DECLARATION

This is to certify that the thesis titled "Effect of Teff Flour Particle Size and Kneading Time on Dough Rheological Property and Injera Quality" submitted in partial fulfillment of the requirements for the degree of Master program on Food Technology under Chemical and Food Engineering Faculty, Bahir Dar Institute of Technology. It is a record of my original work that has never been submitted to this or any other institution for any other degree or certificate. I am humbled by the help and assistance I received during this investigation. All sources of materials used for this thesis have been acknowledged.

Berhanu Regassa	[signature]	14/07/2023
Name of the Candidate	Signature	Date

DEDICATION

This piece of work is dedicated to my father (Regassa Jima), my beloved wife (Bilise Fekeda) and my children (Firaol and Sifan). My father strongly inspiring me throughout my study starting from lower grades whose blessing and unconditional love are acknowledged. Bilise and my children (Firaol and Sifan) were patiently awaited me during MSc study and thesis development. Bilise is very far beyond just wife as she relentlessly offered me all the help I needed to successfully complete my MSc. My beloved kids Firaol and Sifan; let this little work (M.Sc. Thesis) and my achievement is your source of inspiration in all your future endeavors and a reminder that strong commitment and purpose-driven life pay off.

ACKNOWLEDGEMENTS

My Thesis would not come to this stage without GOD's good will and permission that I will praise my greatest and deepest gratitude for the **almighty GOD** who is the entire source of all knowledge and wisdom endowed me and has done all for me through the ups and downs of the whole process.

This work bears fingerprints of several people who contributed to the study at different stages. Mr. Agimassie Agazie, the advisor of mine holds the lion's share that I would like to give my deepest appreciation, gratitude and sincere thanks for his support, guidance, scientific advice, and constructive comments throughout this work. Despite the challenges, he played an amazingly key role in keeping this Thesis on track.

I would like to thank Bahir Dar Institute of Technology, Faculty of Chemical and Food engineering instructors for their contributions in different ways throughout this work and study of my M.Sc. I would like to thank Mr. Asrat Otoro and Mr. Yeshiwas Ayehu for being good friends with invaluable and timely help during laboratory analysis. I would like to thank Addis Ababa Science and Technology University, for allowing me to use the laboratory facilities at the department of food engineering during rheological and pasting property analysis. I would like to express my appreciation to Adet Agricultural Research Center for providing me variety of teff grain sample for the research work.

I would like to thank the financial support from the Bahir Dar Institute of Technology and Food and Beverage Industry Research and Development Center. I would like to acknowledge and appreciate the support of the project team lead by Dr. Mesfin Wogayehu and all members for their unending contributions.

Finally I would like to thank my family, all my friends and colleagues, who appreciated my work and contributed a lot in one way or another, especially Miss. Yodit Fikadu for her support during product formulation and laboratory work. My special thanks go to my beloved wife, Bilise Fekeda for her love, support, patience and constant encouragement including keeping our house and caring our kids alone during my study period.

ABBREVIATIONS AND ACRONYMS

AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
BiT	Bahir Dar Institute of Technology
CHO	Carbohydrate
CIE	Commission International Eclairage
cP	Centipoise
CRD	Completely Randomized Design
CSA	Central Statistical Agency
CFU	Colony Forming Unit
FAO	Food and Agriculture Organization
FPS	Flour Particle Size
GAE	Gallic Acid Equivalent
HSD	Honestly Significance Difference
OAC	Oil Absorption Capacity
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
ppm	parts per million
PSI	Particle Size Index
rpm	Revolution per minute
RVA	Rapid Visco Analyzer
SP	Swelling Power
TA	Titratable Acidity
TPC	Total Phenolic Content
WAC	Water Absorption Capacity
WAI	Water Absorbing Index
WHO	World Health Organization
WSI	Water Solubility Index

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF TABLES IN THE APPENDICES

LIST OF FIGURES IN THE APPENDICES

ABSTRACT

Teff is a cereal grain which is used to make injera, a staple food of Ethiopians. Injera with appropriate quality and consistency is crucial that all consumers demand on the streamlined quality parameters. Because of inadequate process parameters, injera quality is usually a serious problem. Flour particle size and dough kneading time are among the factors that can affect injera quality. This research was aimed to investigate the effect of flour particle size and kneading time on functional, physicochemical, dough rheological property and injera quality. Tseday variety of teff grain was used for the study and milled into different particle sizes. The flour particle size was evaluated by passing through sieve size of 180µm, 355µm and 500µm. Flour particle size of fine (0-180µm), medium (181-355µm) and coarse (356-500µm) was obtained 30.68%, 42.54% and 25.31%, respectively. Mechanical dough kneader was used to knead the injera dough with 4, 8 and 12 minutes with constant and moderate kneading speed of #K_2 (164rpm). The physicochemical and functional properties of flour and microbial and sensory qualities of injera samples were analyzed using standard methods. Higher water and oil absorption capacity were observed in fine flour particle size (1.25g/g and 2.59g/g as compared to coarse flour particle sizes (1.08g/g and 1.77g/g, respectively). Fine particle size of teff flour had loosely packed structure and thus the bulk density of fine flour particle size is (0.68g/mL) lower than coarse flour particle size (0.77g/mL). The L value of flour color was ranged from 75.08 to 81.02 from coarse to fine due to the presence of bran fractions in coarse flour. Observations from pasting properties revealed 1056, 1030, 968 and 1067 peak viscosity for fine, medium, coarse and control flour respectively. Whole teff flour showed significantly ($p<0.05$) lower values of G'(400.23) and G"(246.84) and higher Tan δ (0.54) than treatment flour samples. This indicated the mechanical rigidity of the dough which is preferred for quality injera. The proximate composition of treatment flour samples varied from 8.79-9.05% (moisture), 9.12-11.27% (protein), 2.54-3.00% (fat), 2.02-2.61% (ash), 1.92-3.31% (fiber) and 71.69-74.11% carbohydrate. Proximate composition of injera samples varied with different flour particle sizes and dough kneading times. Accordingly moisture, protein, fat, fiber, ash, carbohydrate and Total energy of all treatment samples ranges from 5.90-6.45%, 10.32-12.82%, 1.18-1.73%, 2.01-3.37%, 2.40-2.73% 73.68-77.66% and 356.81-364.25%, respectively. Significantly higher contents of phytochemicals and minerals were perceived on coarse flour particle size injera than fine due to the presence of higher concentration of grain's outer layers in coarse flour. The bacterial load of injera was detected in samples of injera made from fine flour that had been kneaded for 12 minutes on day five of storage with a value of 4.54log cfu/g Injera samples had a yeast/mold load that ranges from 1.77 to 5.08log cfu/g from day one to day five storage. The highest overall acceptability of injera by the panelist was formulated from fine flour particle size with 12 minute dough kneading time having the score of 4.66. In general, fine flour particle size with 12 minute dough kneading time resulted in desired nutritional and injera quality.*

Keywords: Injera quality, Kneading time, Particle size, Rheological property, Teff flour

1. INTRODUCTION

1.1. Background

Ethiopia is the country where a wide variety of cereal grains are cultivated and utilized to different food types. Teff [*Eragrostis teff (zucc.)Trotter*] is one of an ancient cereal grain cultivated over a wide range of environmental conditions mostly in the highlands of the country (Baye, 2014) and processed to different traditional foods. Injera is one of the fermented flat bread products made commonly of teff flour (Anberbir *et al.*, 2023). Injera that is considered good by the consumer is soft, fluffy, rich in eyes and rollable without cracking (Girma *et al*., 2013b). It has a distinct flavor that is slightly sour (Yetneberk *et al*., 2004). The surface of injera has evenly spaced holes that form a honeycomb-like structure due to carbon dioxide gas production during fermentation and baking (Woldemariam *et al*., 2019). It has a smooth and shiny bottom surface (Yasin, 2021). Teff grain is preferable to make injera that fulfill these requirements in addition to gluten free and rich in nutrient.

Teff grain is always milled to whole grain flour and fermented before being consumed as injera. Depending on the mechanical forces and temperature used during the grinding process, different milling or grinding procedures have been found to yield distinct flours with different particle sizes (Kadan *et al*., 2008). The main factors impacting the particle size of teff flour in the milling process include milling equipment and procedures, such as mechanical force and non-standardized milling intensity (Assefa *et al*., 2018). The process of reducing flour particle size increases the ratio of surface area to volume of a food material (Ahmed *et al*., 2014).

Kneading is one of the common crucial unit operation in mechanical dough processing (Esselink *et al*., 2003). The main goal of kneading is to get a homogeneous mixing of raw and auxiliary ingredients and obtaining dough with a viscous-elastic structure and qualities *(*Canja *et al*., 2014). During kneading, a quantity of air is introduced into the dough, which is critical for the dough rheology as well as the end product's quality.

The rheological properties of dough and product quality are affected by the flour particle size and dough kneading time (Coţovanu & Mironeasa, 2022). The size of the particles can have a big impact on functional and physicochemical parameters such as water and oil absorption, viscoelastic and pasting properties (Rao *et al*., 2016). Particle size influences the pace at which liquid is absorbed into the flour. Fine flour absorbs liquid faster than medium or coarse flour, which affects dough preparation (Lin *et al.*, 2020). When grain is milled into flour at various processes of extraction, the components of the grain, such as bran and endosperm, are lowered below whole flour. Refined flour contains lower fibers, phytochemicals, vitamins, and minerals than whole-grain flour (Vignola *et al*., 2016).

The visco-elastic characteristics and their rheological parameters of dough are affected by the mixing time of dough preparation. They are strongly related to the quality of injera. The existence of specific components (proteins, starch, and fat) as well as the structure of the injera based on interactions between the ingredients, as well as preparation methods, have the biggest impact on the rheological features of foods. Dough rheological parameters reflect the quality of food items to a considerable extent, allowing the development of products with features that customers find acceptable (Abebe & Ronda, 2014). The dough is a sticky mass made by combining water, teff flour and Ersho in a mixing bowl (Neela & Fanta, 2020). The quality of dough is determined by the perfect mixing of all ingredients in the correct ratio in precise equipment, which ultimately affects the final injera qualities. The addition of water and the formation of suitable mixing operations are the first steps in the dough development process. The characteristics of dough rheological properties are influenced by the particle size distribution and mixing time and ultimately affect the quality of injera (Fikre *et al*., 2019).

Tef injera-making holds a considerable economic and social interest that demands a detailed investigation of the process variables that influence the quality of the final product (Y. Assefa *et al*., 2018). The intrinsic and functional properties of flour are greatly influenced by particle size, which in turn influences the quality of the product obtained from that flour. Sieving flour can result in samples with homogeneous particle size distribution, which can lead to the production of products with improved functional qualities (Bala *et al*., 2020). However there are limited findings on the effect of flour particle size and dough kneading time on the rheological characteristics of dough and its final product qualities. In this study, therefore, knowing consistent action of process parameters (flour particle size and dough kneading time) creates consistent quality of injera.

1.2. Statement of the Problem

Teff grain is an increasingly important dietary component for individuals because of its nutritional benefits. It has been used by the people to prepare their basic meals or as a stream of income. Most Ethiopians relies on injera which is produced from teff flour or other grains based on their affordability. Injera with the required quality and consistency is critical for all consumers. However, the quality of injera is always a serious issue at house hold as well as commercial levels due to poor optimized process parameters. The overall quality of injera is influenced by processing variables such as flour particle size, dough kneading time and viscosity. Adequate particle size distribution among teff flour and dough kneading time are important factors in achieving consistent quality when processing injera. To commercialize injera, it is crucial to manufacture required quality of injera that is acceptable to consumers. Kneading injera dough is done by hand, which is enervating, especially for mass producers, and unsafe for product quality.

Even though injera is prepared in most Ethiopian homes and injera bakeries, teff flour particle size and dough kneading time has not been optimized and improved based on the final product quality. Traditionally, particle size of the teff flour at milling is checked manually with hand and mixing time of dough has no fixed or standard time and that may affects the rheological properties of dough. The particle size of the milled flour and the mixing time of dough should be optimized to maintain the quality of injera that meet the consumer`s preference. Consumers prefer injera with quality features such as a pliable, honeycomb-like surface, fluffy, soft, and sour taste, which should be maintained by process parameters.

Dough prepared from different particle size and different kneading times have different dough purity that affects the final product. Optimized particle size with proper dough kneading time can manage the water absorption and rheological properties as well as determine the quality of the final product. Baking of injera with appropriate quality help consumer to get demanded product and bakers to produce consistent quality for the increased export demand. Due to the limited work on process parameters to maintain injera quality, it is critically important to investigate the effect of flour particle size and dough kneading time on the dough rheological properties and injera qualities.

1.3. Objectives

1.3.1. General Objective

This research was aimed to investigate the effect of teff flour particle size and mechanical dough kneading time on the dough rheological property and elucidate quality characteristics of injera

1.3.2. Specific Objectives

- To investigate the effect of flour particle size on the bulk density, pasting properties, physicochemical and functional properties of teff flour
- To analyze the effect of flour particle size on the dough rheological properties and physicochemical, sensory properties and microbiological qualities of injera
- To study the influences of mechanical kneading time on dough rheological properties and injera quality attributes (physicochemical, microbial and sensory properties)
- To assess the interaction effect teff flour particle size and mechanical dough kneading time on dough rheological properties and final injera qualities

1.4. Significance of the Study

The finding of this study is valuable in determining the importance of particle size on the rheological properties of dough and its qualities of end products. Particle size will be utilized to predict product quality attributes and to identify optimal teff flour quality. The investigation will show the gap of dough kneading times that contribute a baseline science for developing effective dough rheology and injera quality traits. The study will assure the society to offer optimal particle size of teff flour and kneading time of dough for the required rheological properties of dough and enhanced quality of injera. Rheological properties of the dough are investigated to see its effects on the quality of injera. The following elements make it crucial to carry out this research on the impact of flour particle size and dough kneading time on dough rheological characteristics and injera quality.

- The study can give information for injera producers as it helps to obtain a better understanding of flour particle size and dough kneading time for making quality injera.

- The study will give more information for teff millers that produce optimized flour particle size for acceptable injera.
- This research will assist the injera producers to use mechanical kneader that have fixed kneading speed and manageable kneading time to exclude hand kneading of dough to maintain quality injera.
- For those who desired to investigate the related concerns, the general information provided by this study will be used as a reference.

1.5. Scope of the Study

This study was limited by examining the teff flour particle size and optimizing of dough kneading time on mechanical kneading unit operation at a constant rotational speed for dough rheological properties that determine the quality of final product. Functional and physical properties of flour and injera, rheological properties of dough, proximate composition of injera, microbial load of injera as well as its sensory analysis were analyzed. The mineral content of flour and injera as well as phytochemicals that are affected by flour particle size and dough kneading time were assessed.

2. LITERATURE REVIEW

2.1. Teff Production and Utilization in Ethiopia

The agricultural product production is subsistence in Ethiopia by a considerable amount. Cereal crops are the major crops that covered almost all the regions used to utilize local food and used as income generator for the farmers. Warm-season annual cereal teff (Eragrostis teff) is one of the underutilized crops that can support crop diversification and food security. One of Ethiopia's most important and native cereal crops is teff (Kebede & Korji, 2017). It is a special crop that can withstand a variety of weather circumstances and has been used as food and diet supplements for the majority of Ethiopians. Teff has exceptional nutritional qualities that will satisfy the needs of consumers who are health-conscious. Additionally, it is a low-risk crop that can withstand numerous biotic and abiotic challenges. Ethiopia is the only nation that has made teff a staple crop (Baye, 2018), and produces most of it at the moment. However, Ethiopia's teff production and value chain heavily rely on conventional methods, and the government's export prohibition restricts the teff market (Central Statistical Agency (CSA), 2020). Based on the Ethiopian Central Statistics Agency, the current teff productions in Ethiopia is 56,143,388.01 in quintals (CSA, 2022). Teff is mostly used to make the Ethiopian bread injera, though it has lately been used to make gluten-free goods including cake, cookies and bread (Zhu, 2018).

2.2. Physical Structure and Chemical Composition of Teff Grain and Flour

Teff grain size is exceptionally small, with a mean length of 0.61-1.17 mm and a mean width of 0.13-0.59 mm, resulting in an average thousand kernel weight of 0.264 gram (Bultosa, 2007). The grains of teff seeds are oval in shape. Teff cultivars have been identified and classified based on the color of the grains and inflorescences, inflorescence ramification and plant size. Depending on the variety, teff can range in color from white (ivory) to dark brown (black). There are three major groups in Ethiopia: white (nech), red (quey), and mixed (sergegna) (Abewa *et al*., 2019). Wholesalers commonly classify white teff into two categories: very white (magna) and white (nech). When teff whole grain is finely processed, the color difference between the flour and the whole grain becomes less evident. This could mean that the pigmenting components in brown teff grains are concentrated mostly on the grain pericarp (Baye, 2014).

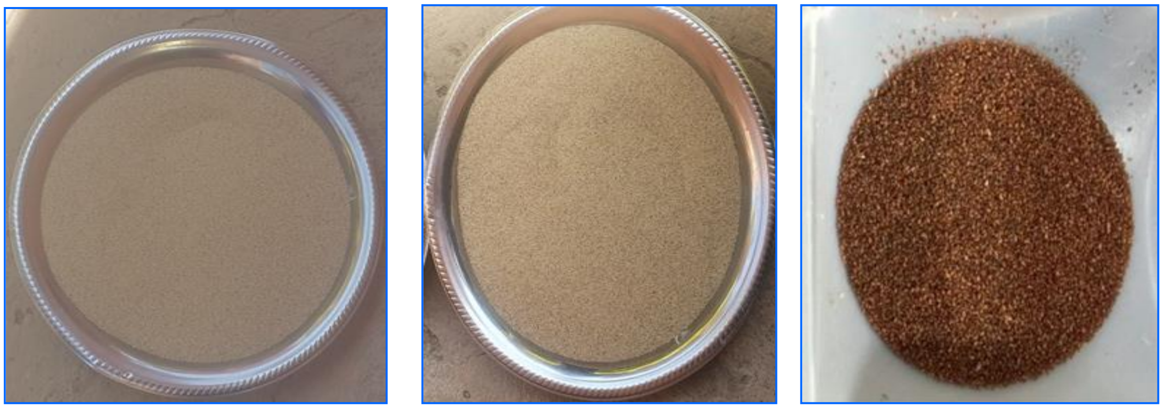


Figure 2.1. Physical structure of teff grain

Source: Bultosa, (2007)

The seed of teff is protected by the thin outer coat, pericarp, which forms bran. The inner surface of bran in red and brown (mixed) teff types has pigmented material with increased polyphenols and tannins similar to those found in finger millet and sorghum *(McDonough et al.*, 2000). The authors add as the endosperm makes up the majority of the teff seed, with protein bodies that are unevenly dispersed mostly in the outside section and starch granules originate in the central part of the endosperm. The starch granules present in teff are 2-6µm in diameter, smooth, and polygonal in shape, and account for the majority of the carbohydrate percentage in teff (Bultosa *et al*., 2002).

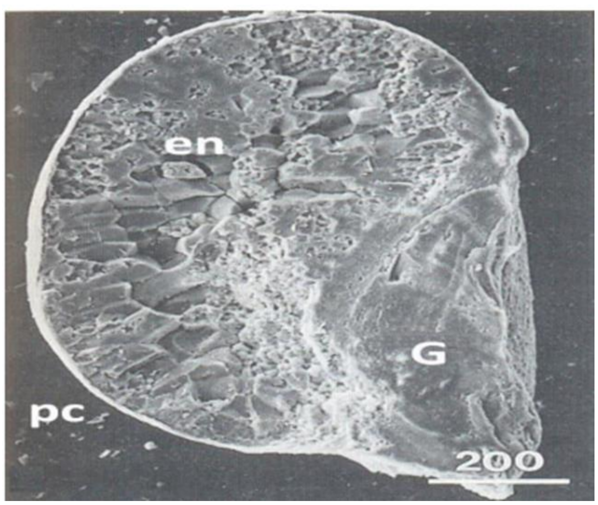


Figure 2.2. Scanning electron micrograph of a halved teff grain

Source: Parker & Faulks, (1989)

Where, pericarp (pc), the starchy endosperm (en), germ (G) with outer horny region and mealy center (arrowed), and relatively large embryo (em)

Teff is one of the popular cereal crops for general health consumption; however, due to consumer inexperience and lack of interest in teff, Ethiopians have long believed their crop is of lower quality (Yetneberk *et al*., 2005). Numerous investigations have been conducted on the post-harvest qualities and composition of teff nutrition. In countries other than Ethiopia, the development of novel teff-based food products is accelerating because of the gluten-free nature of the grain (Baye, 2014).

The chemical composition of teff grain varies greatly and influenced by environmental factors such as soil, variety, and fertilizer in cereal crops. Teff's value stems mostly from its appealing nutritional profile and the absence of gluten present in other common cereals such as wheat, barley, and rye. The demand for gluten-free goods is increasing these days among people who have celiac disease or other types of gluten sensitivity (Hopman *et al*., 2008). Teff has a better nutritional value than many other cereals and mostly used to make injera, which is its major purpose (Bultosa, 2002, do Nascimento *et al*., 2018). It contains high in phosphorus, magnesium, iron, and calcium. The seeds of Eragrostis teff have a high nutritional value, with carbohydrates (57.27%), protein (20.9%), essential amino acids (8.15 %) with major leucine and lysine (1.71 and 1.35%, respectively), vitamin B1 (1.56%), potassium and calcium (32.4 and 9.63%, respectively. According to a proximate analysis of El-Alfy *et al* (2012) the seeds provided 22% (w/w) fixed oil rich in unsaturated fatty acids (72.46 %), with oleic acid leading the way (32.41%) and linolenic acid following closely after (23.83%). The teff grain is made up of complex carbohydrates with 80% of total weight base. It is a starchy grain with a starch content of nearly 73% (Bultosa, 2007). It has an average crude protein level of 8 to 11 percent, which is similar to other common grains like wheat. Teff grain has high amount of calcium, Iron, zinc and fibers relative to other cereal crops. According to Baye (2014). Teff has 17-178 mg/100g calcium for different variety of teff, 7.63 mg/100 g iron, 3.63 mg/100 g zinc and 184 mg/100 g magnesium. The author also identified the phytochemicals of teff flour as 140mg/100g total phenols, 682 mg/100g phytates and 16 mg/100 g condensed tannins. Yegrem et al., (2019) reported the Tseday variety of teff flour contain 10.71% moisture content, 2.46% ash, 12.50% protein, 2.96% fat and 3.15% fiber. They include 15.87, 3.24, 127.97 mg/100g of iron, zinc and calcium, respectively.

The mineral content of teff injera increased with increased fermentation time. Iron content ranged from 18.83-19.18 mg/100g, zinc content ranged from 14.27-14.66 mg/100 g zinc and calcium content ranged from 123.86-136.86 mg/100 g as fermentation increased from 24-72 hour. Antinutritional content of injera decreased with increasing fermentation time as tannin decreased from 0.015 to 0.008 and phytate decreased 295.46-234.46 mg/100 g on fermentation increased from 24-72 hours (Mihrete, 2019). Asres et al., (2018) added phytate of teff decreased from 541.45-440.89 mg/100 g and tannin decreased from 0.87-0.74 when fermentation time increased from zero to 72 hour.

2.3. Overview of Teff Grain and Flour Particle Size

Particle size (granularity) is a quality control parameter of cereal flour after it is milled. The particle size of granular materials is commonly referred to as diameter, which is usually measured by geometric methods (microscopy, or by sieving a representative amount of sample) (Saravacos & Maroulis, 2011). To evaluate the particle size distribution of a granular material, engineers frequently utilize particle size analysis, also known as sieve analysis. The material's performance in usage is frequently critically impacted by the size distribution. Depending on the precise procedure, a sieve analysis can be done on every type of grain flour, a wide range of produced powders, grain, and seeds, down to a minimal size.

Sieve analysis is a technique for determining a material's particle size distribution. By running the material through a series of sieves with varying mesh sizes, the method separates tiny particles from coarse particles, effectively fractionating particles within predetermined sieve bin sizes. This mass fraction of particles is weighed and quantified in order to create a cumulative distribution. The most common and well-known method for characterizing particle size distributions is sieve analysis. Wet sieving and dry sieving are the two forms of sieve analysis that can be performed. Wet sieving is appropriate for particle sizes ranging from 20 μm to 3 millimeters, whereas dry sieving is appropriate for particle sizes ranging from 30 microns to 125 microns (Barak *et al.*, 2014).

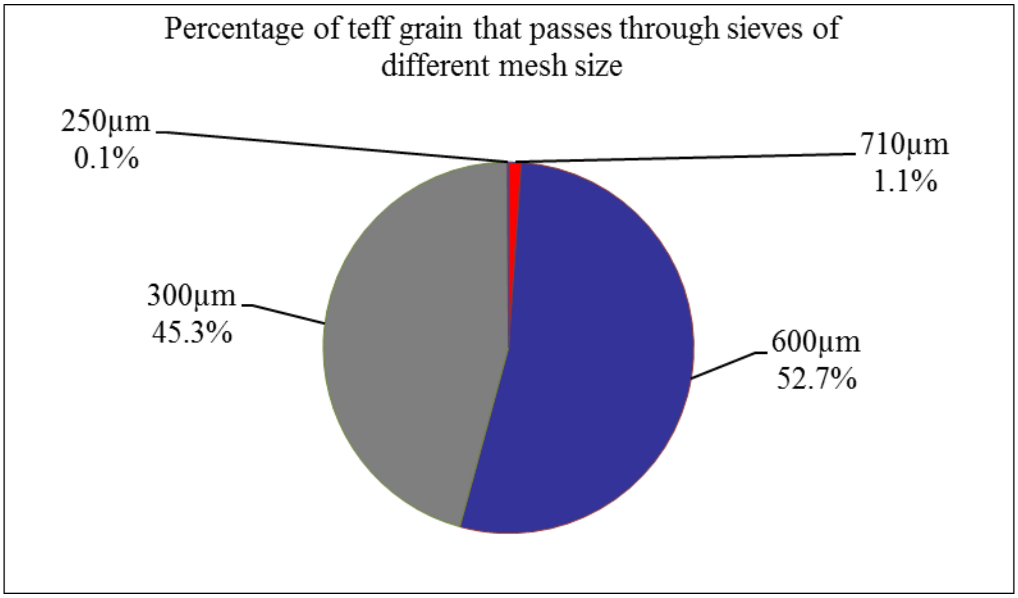


Figure 2.3. Percentages of teff grain passes through sieves

Source: Bultosa, (2007)

Flour samples made through milling and grinding contain a heterogeneous combination of different particle sizes. The intrinsic and functional properties of flour are greatly influenced by its particle size, which has implications for the final product's quality. A flour or powder's complete description is provided by the particle size distribution. The production of a product with higher functional qualities can be achieved by sieving flour to obtain samples with a homogeneous distribution of particle sizes (Bala *et al*., 2020).

Sieve analysis is a common engineering process or procedure for determining the particle size distribution of a granular material. The size distribution of a material can have a significant impact on how it performs in usage. Depending on the exact procedure, a sieve analysis can be performed on any type of grain flour, a wide range of produced powders, grain, and seeds, down to a minimum size. It is perhaps the most used particle sizing procedure since it is so simple (Munteanu *et al*., 2015). Powders with particle sizes bigger than 200micrometers are free flowing, however small powders are more difficult to flow due to cohesion. The particle size distribution is an essential factor in the look of a product. The overall bulk qualities of the food item, such as visual texture and density, as well as color, are influenced by particle size (Abebaw, 2020).

Pang et al., (2021) studied the effects of wheat flour particle size on flour physicochemical properties and steamed bread quality and conclude as the physical and chemical qualities of wheat flour and its ultimate product are heavily influenced by particle size. The impact of five milling intensities on the physicochemical attributes and quality of wheat flour for skimmed bread was investigated in this study. They discovered that increasing the milling gap lowered the particle size of wheat flour while increasing the damaged starch concentration.

Regarding to evaluating the quality of grains and flour, flour color is a key factor. A crucial quality indicator for millers, flour color is measured and reported using sophisticated laboratory equipment by coordinate of CIE *L***a***b** scale. Colorimetric color measurement of flour uses a numerical system to assess a sample's "chromaticity," or hue, on two separate scales, one for green-red (a*) and one for blue-yellow (b*), each running from -60 to +60. A color with a high L* value is bright, and a color with a high b* value is more yellow. Flour color is controlled by the grain endosperm color, particle size and flour ash concentration often altering the color of the completed product (Barretto *et al.*, 2021).

Due to the size of the flour particles, measuring and assessing flour color visually is challenging. Light is reflected back to the source, detector, or observer when it contacts the surface of an extraordinarily fine particle mass, providing the impression of a smooth sample surface. A perceived and measured increase in light reflected from the sample suggests a lighter look of color. Since the surface becomes rougher with increasing particle size, more light is reflected off in directions other than the source, observer, or detector. A darker color appearance is indicated by a perceived and measured decrease in light reflected by the sample. The size of the flour particles may influence diffusion, whilst the color of the darker bran particles may influence absorption.

2.4. Effect of Kneading Time on Dough Rheology and Injera Quality

Y. Assefa et al., (2018) studied the effect of kneading time on the quality of injera. They reported that the color, quantity of eyes, taste, and odor of injera formed from dough obtained under various kneading conditions were different. The sensory qualities, such as texture, eye size, distribution, top and bottom surfaces, and overall acceptability, were, considerably influenced by the kneading circumstances. This could be explained by the relation between kneading and gas production. Kneading or remixing the dough encourages the release of large gas bubbles, resulting in a more even distribution of bubbles throughout the dough, which ultimately improves the product's quality. Physical, colloidal, and biochemical processes all contribute to the production of the dough with its specific structure and rheological qualities, with the physical and colloidal processes playing the most vital function (Rosell, 2011).

Maltose was the largest sugar concentration initially in both milling and kneading levels, followed by glucose and fructose. Maltose breakdown followed a similar pattern in all mill types investigated as fermentation progressed. However, both mills and kneading speed-time combinations showed significantly differing phytates/mineral molar ratios of the flours (Assefa *et al*., 2018). Flavonoids, total phenolic, and phytates levels are all affected by the kneading time-speed combinations, according to the study of Y. Assefa et al., (2018). In addition, the overall acceptability of injera was reported by the kneading process. Changes in kneading conditions had a substantial impact on injera quality features such as texture, eye size, distribution, top and bottom surfaces, and overall acceptance. This could be explained by the correlation between kneading and gas production. Kneading or remixing the dough encourages the release of large gas bubbles, resulting in a more even distribution of bubbles throughout the dough, which ultimately improves the product's quality.

2.5. Functional Properties of Teff Flour

The structure, quality, and texture of a food, as well as its nutritional content, acceptability, and (or) appearance, all relate to its functional properties. The organoleptic, physical, and/or chemical aspects of a food are frequently what determine its functional characteristics. Additionally, solubility, water retention, foaming ability, elasticity, absorptive capacity for fat and foreign particles, emulsification, hydration (water binding), viscosity, cohesion, and stickiness are examples of functional qualities of flour (Ch, 2013). Abebe et al. (2015) studied the functional properties of teff flour and compared with wheat and rice flour. Accordingly flour water absorption capacity was significantly affected by both type of teff cultivar and mill type. Teff flour's higher fiber content as a whole meal may explain its increased water-binding capacity when compared with refined rice and wheat flour (Collar & Angioloni, 2014).

The water solubility index of teff flour is higher than other cereal grain flour. Yetneberk et al., (2005) reported that increasing proportion of teff flour to sorghum for quality of injera increase the water solubility index. The increase in water solubility index corresponded with the observation that, after mixing, teff dough tended to be stickier than sorghum dough, and water-soluble components in the teff flour could have positively changed the dough rheology and texture of injera (Yetneberk *et al*., 2005). Finally higher water solubility index yielded softer, fluffy, and rollable injera in the investigation of injera-producing possibilities of sorghum varieties.

2.6. Bulk Density

Weight per volume of flour is known as bulk density. The flour expansion and porosity of goods are determined by bulk density (Bala *et al*., 2020). This study implies that as the size of the grass pea flour particles reduced from 249μm to 74μm, the bulk densities of the various flour samples dropped from 460 kg/m^3 to 420 kg/m^3. This means flour particle size and bulk density are directly proportional that coarse particle size have higher particle size when compared to fine particle size. The flour's larger bulk density suggests that this product may also need a denser packing material. Bulk density provides details about a product's porosity and should guide the selection of the package and its layout (Odedeji & Adeleke, 2010).

2.7. Pasting Properties

The phenomena of granular cooking, swelling and total disruption of granules are known as starch's pasting properties. It has been used to measure cold-swelling of 'cooked' components, raw component paste during testing, and overall viscosity, which shows the degree of starch dextrinization (Tsegaye, 2020). The properties of the swollen granules and the soluble ingredients leached out of the granules work together to influence the viscosity parameters during pasting. The addition of absit to the fermented batter raises the viscosity of the batter and improves its gas-holding capacity (Torres-Gallo *et al*., 2021). The texture, digestibility, and final use of the food product are all affected by these changes. Pasting temperature is about 50^oC-90^oC and absit preparation for dough binder in injera preparation takes the temperature of 45-70^oC which is in the range of pasting starch. It has been customary to employ the BVA (Brabender viscoamylograph) and RVA (Rapid visco-analyser) to examine the pasting properties of pulse starches. Different concentrations and BVA or RVA techniques have been used to determine the pasting curve of pulse starches (Niazi *et al*., 2012).

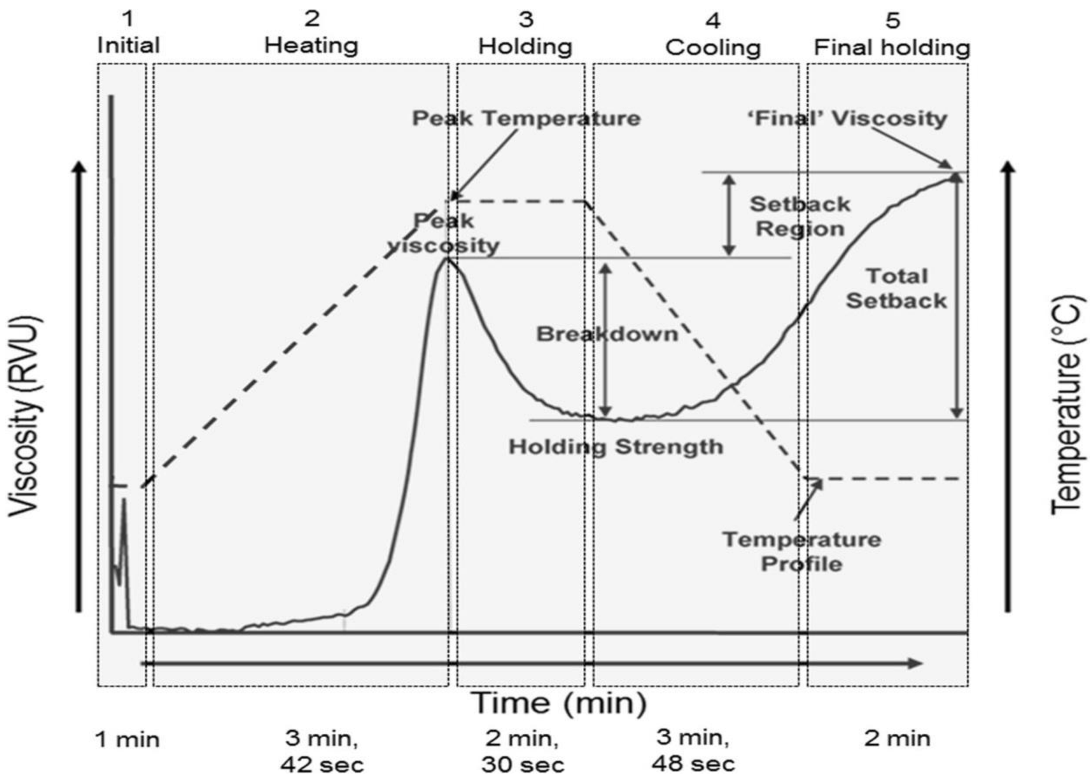


Figure 2.4. A typical RVA pasting profile of a cereal (using the standard profile), indicating the main parameters measured during analysis.

Source: Balet et al., (2019)

2.7.1. Peak Viscosity (PV)

Peak viscosity is the maximum viscosity that starch can reach throughout the gelatinization process (50-95^{o}C), and it represents the starch granule's ability to thicken and bind water. It occurs at the point of equilibrium between granule rupture, alignment, and swelling, which increases viscosity or decrease of its viscosity (Shimelis *et al*., 2006). The PV emphasizes the stability of pastes, which are created when gelatinization occurs during food preparation. It also reveals the expected viscous load to be faced during mixing and redirects the extent of granule swelling (N. Singh *et al*., 2017). The peak viscosity of teff flour from different mill type and variety teff grain were reported as 1336-1676 (mpas) Abebe, Ronda, et al., (2015) and 1544-1768 (mpas) by Y. Assefa et al., (2018).

2.7.2. Trough Viscosity (TV)

The ability of the paste to resist breaking down while cooling is measured by its trough viscosity, which is the lowest value in the constant temperature phase of the flour's RVA pasting profile (Ayo-Omogie & Ogunsakin, 2013). Gelation occurs with junction zone formation primarily through hydrogen bonding, re-associating the hydrated and dispersed starch molecules, and may vary with the botanical sources of the starch, amylose content and formation of amylose-lipid complexes, amount of water, other ingredients like proteins, and cooling temperature (Bultosa *et al*., 2008).

2.7.3. Break down Viscosity (BDV)

The breakdown viscosity of flour can be used to describe its paste stability. When characterizing the stability of starch granules during heating and paste consistency, breakdown (BDV) is a crucial characteristic. It is the difference between the peak and trough viscosities (Patindol *et al*., 2005). The starch sample's ability to withstand heat and shear stress during cooking would be reduced due to the high viscosity breakdown. The resistance of starch to thermal pasting is measured by breakdown viscosity. Lower heating tolerance is found in flour with higher breakdown viscosity. Disrupted granules and linear molecules leak into solution during breakdown (Wang *et al*., 2020).

2.7.4. Final viscosity (FV)

The final viscosity reveals the starch's capacity to create a viscous paste after the cooking and cooling stage at almost $50^{o}C$. The starch molecules in the granules were restructured and retrograded. The fact that it has the highest ultimate viscosity among starchy flours indicates that it is suitable for use as a food thickening agent in food applications (Shafie *et al*., 2016).

2.7.5. Set back Viscosity (SBV)

Setback viscosity is the indication of the starch retrogradation tendency after gelatinization and cooling at 50°C. Setback viscosity can be calculated by subtracting peak viscosity from final viscosity. Low setback viscosity, which showed a slow rate of starch retrogradation, and amylose molecule re-association were the main causes of the viscosity variations during cooling (Shafie *et al*., 2016).

2.7.6. Pasting Temperature (PT)

The minimum temperature needed to cook a starch is known as the pasting temperature. It refers the temperature at which heating causes the viscosity of starch to start to increase (Kumar & Khatkar, 2017). The initial increase in viscosity happened as proteins and starch granules started to absorb water and swell as the temperature grew gradually. The gelatinization properties of the starch source were correlated with the temperature at this moment generally referred to as the pasting temperature. It is the temperature at which viscosity first begins to increase. According to the amount of amylose and amylopectin in the teff cultivars, the ingredient's ability to resist swelling was indicated by the higher pasting temperature (MATHEW, 2007).

2.7.7. Pasting Time (Pt)

Pasting time is an indicator for the shortest amount of time needed to cook flour. Peak time refers to the instant in a minute when viscosity peaked (Gebru *et al*., 2019).

2.8. Dough Rheological Properties

The study of how materials deform, flow, or fail when force is applied is known as rheology. It is the study of how substances flow and deform, with a focus on how they behave in the transition zone between solids and fluids. Materials that are deformed and subjected to shearing forces are known as visco-elastic complexes, and their behavior is described by rheological properties. The viscosity of starch is its primary characteristic. Texture, transparency or clarity, shear strength, and the propensity for retrogradation are other rheological parameters. These attributes all have significant functions in the commercial applications (Berski *et al*., 2011). Furthermore, rheology tries to describe a link between the stress applied on a material and the consequent deformation and/or flow. Force and deformation as a function of time are used to determine rheological characteristics (Komlenić *et al*., 2012).

Dough rheological and final product characteristics are affected by kneading at different times. Excessively long mixing times result in dough that is softer, less uniform, and stickier than dough that has been combined properly. Over mixing, on the other hand, has a strong relationship with the type of flour used (Gómez *et al*., 2011). The rheological parameters of foods are used in food quality control and processing, such as energy input calculations, process design, equipment selection, and, in particular, the selection of heat exchangers and pumps (Mirza Alizadeh *et al.*, 2020). Rheological characteristics of dough are concerned with the properties of substances in terms of particle interactions, and they apply to both solid and liquid entities. The viscosity and elasticity of dough are affected by the mixing period of dough preparation, which is referred to as visco-elastic characteristics, and their rheological parameters are strongly related to the form of injera. The existence of specific components (proteins, starch, and fat) as well as the structure of the injera based on interactions between the ingredients, as well as preparation methods, have the biggest impact on the rheological features of foods. Food rheological parameters reflect the quality of food items to a considerable extent, allowing the development of products with features that customers find acceptable (Abebe & Ronda, 2014). The rheological qualities of the dough play a critical role in the behavioral evaluation of dough processing (water absorption, dough development time) and the end product quality (Codina *et al*., 2011). Rheological characteristics such as elasticity, viscosity and extensibility are the vital properties for bakery industries in prediction of processing parameters of dough quality and end products. This rheological properties change throughout injera baking process.

G″ is the loss modulus, which describes the amount of energy lost by the viscous deformation of the material and is positively correlated with viscosity; G′ is the storage modulus, which characterizes the energy stored in the material after elastic deformation, and is positively correlated with the elasticity of the object. Tan δ is the ratio of G″ to G′, which can be used to determine whether a material tends to be viscous or elastic based on the size of its relative viscous and elastic moduli (Skendi *et al*., 2011).

Studies have demonstrated the potential for grain starches to be damaged for a variety of reasons, particularly during milling (Ali *et al*., 2014) (Abebe, Collar, *et al*., 2015). The overall rheological characteristics and the end products are affected by these damaged starches. Both storage modulus (G′) and loss modulus (G″) increased as particle size decreased, and both reached their maximum when the flour had the smallest particle size. This demonstrates how dough becomes more elastic as the particle size decreases (Pang *et al*., 2021).

2.8.1. Viscosity of Dough

Viscosity of injera dough is the resistance of dough to movement when shear forces are applied to it. During dough kneading the viscosity of injera dough is analyzed. Viscometer measures and records the resistance to deformation over time of a flour/water slurry or batter while it is subjected to heating and cooling cycles (Pauwels, 2013). Teff starch pastes or batter exhibit non-Newtonian viscosity behavior, or stress thinning, which is a characteristic of nearly all starchy foods. Teff starch paste demonstrated a stress maximum that will decrease when temperature varies between 25 and 90 degrees Celsius. That greater at 25°C than at 90°C is evident. The creation of hydrogen bonds at lower temperatures stabilizes and strengthens the matrix, causing the fluctuation of stress and the stiffness of gels.

2.8.2. Elasticity of Dough

The degree to which a dough piece will attempt to regain its original shape after a deforming force has been removed is known as elasticity. The dough should have a minimum of elasticity to avoid misshaping caused by “spring-back” after sheeting and moulding in the first stage of dough mixing (R. P. Singh & Heldman, 2014).

2.8.3. Extensibility of Dough

Dough extensibility is the ability of dough to stretch or deform. Deforming forces include sheeting and moulding pressures as well as gas pressure from yeast fermentation. Dough should have enough extensibility (and minimal elasticity) to yield significant dough expansion during proofing and baking (M. A. Rao *et al*., 2014).

2.8.4. Resistance to Deformation of Dough

The term dough softness is used to describe the idea of dough resistance to deformation. Dough should have as minimal deformation resistance as feasible. This corresponds to ease of form change during sheeting and moulding, avoiding damage to the rather fragile gas cells introduced during mixing (R. P. Singh & Heldman, 2014). Rheometer is the instrumental material used to measure the rheology of material which shows rheological properties.

2.9. Preparation of Teff Injera

Injera is a fermented, soft, circular flatbread with little bubble structures or eyes (honey-comb-like openings) on its top surface caused by CO_2 creation and escape during fermentation and baking. It is mainly made from teff grain or a combination of several of cereals such as barley, sorghum, maize, millet and wheat depending on availability and abundance (Bultosa, 2007). The most tolerable (sensory) injera should have a lot of eyes, be softer, thin, rolable, and have a sour taste because of the fermenting process (Girma *et al*., 2013b). Commercial mills transform the grains used in injera recipes into flour (disc mills are the commonly used). It is customary to combine one part flour with two parts water and around 16% (w/w) "ersho" by weight of flour. The flour, water, and ersho are properly mixed to produce a thin, watery paste, which is then fermented for 2-3 hours (Ashagrie & Abate, 2012). After 2-3 days primary fermentation, a portion of the batter mixed and boiled to produce absit. A prepared absit mixture is returned to the original fermented batter for a 2-hour secondary fermentation and finally, the batter is ready for making injera. Injera is baked by putting roughly about 400ml of batter onto a heated greased plate known as a "metad" (clay injera griddle) and moving it around in a circular motion from the edges to the middle (Neela & Fanta, 2020). The majority of Ethiopian people like and consume injera made from teff on a regular basis due to its softer texture, preferred taste, acceptable color and nutritional value.

2.10. Quality Characteristics of Injera

The appearance, texture, and flavor of injera are all strongly tied to its quality. A typical injera is rounding, soft, porous, Fluffy, and resilient, with uniformly placed honeycomb-like "eyes" on the top, about 6 mm thick and 60 cm in diameter. Injera is remarkable in that it is leavened while not being manufactured from gluten-containing grain (Attuquayefio, 2014). Poor grade injera has huge unevenly spread eyes or small eyes. The former denotes insufficient fermentation, whereas the latter denotes excessive absit in the dough. Injera's backside is usually smooth and lack of eyes. An excellent injera is also spongy and won't crack when folded and excellent grade injera will also have a non-powdery, silky look.

Injera of poor quality is fragile, crumbles readily when handled, and has a powdery, dry, or sticky, rusty brown appearance on the back (Fikre, *et al*., 2019).

Depending on the color of the Teff flour used, injera might be pale, cream, reddish brown, or brown in hue. The most popular injera is whitish or cream in color, with a soft and malleable texture that lasts for up to three days after cooking, is relatively thin, and has evenly spaced medium-sized eyes. The backside of the injera must be smooth, not sticky, and free of rust or burn marks. When touched, good quality injera does not fluff up and adhere to the fingers. To get the right taste combination with the spicy stew, good injera must be slightly sour. Injera should also have a spongy texture. Texture refers to how a substance feels in the hands and mouth as a whole. It affects the flavor release of a food product and contributes to the overall eating experience. Because injera is used as a utensil, its texture is an important quality attribute. The fermentation procedure and the length of time for fermentation have a significant impact on the quality of injera. The flour particle size and dough kneading time are also the basic quality parameters that decide the acceptance of injera for consumer (Abebe, Ronda, *et al*., 2015). The measurement of a food's reaction to pressures including cutting, shearing, chewing, compressing, or stretching is known as instrumental texture evaluation. The viscoelastic characteristics of the food influence its texture.

The inspection of a product (such as meals and beverages) through the evaluation of the properties traceable by one or more of the five human senses such as taste, smell, touch, sight, and hearing is referred to as sensory evaluation. In food science, it is used to honestly evaluate food quality. Because it makes it possible to determine objectively whether or if consumers will accept a novel food product, it is frequently a crucial tool (Piana *et al*., 2004). Color, flavor, texture, number of eyes, eye size, eye distribution, top and bottom surface, and overall acceptability of injera were quality parameters that are analyzed by sensory panelist for injera (Cherie *et al*., 2018).

Abraha & Abay (2017) assessed the sensory qualities of injera produced from a blend of cereals (Teff, barley, sorghum, and maize) in proportions of 100, 75, 50, and 25%. Their findings found that cereal flour mixes in injera had no significant differences in texture, mouth feel, overall acceptability, color, flavor, and the appearance of injera surface eyeballs.

According to the findings of this study, the quality of injera made using teff and other grains was second only to teff. The taste of injera is related with the sweet, sour, and bitter sensations produced in the mouth by injera contact (Ghebrehiwot *et al*., 2016). One of the most essential factors is the appearance of injera, which refers to the quality of the eyes (cells) of the honeycomb-like structure of the top surface of injera generated during cooking owing to escaping CO_2 bubbles (Yetneberk *et al*., 2005).

Y. Assefa et al., (2018) studied the effect of teff mill type (hammer mill, disk mill, and blade mill) on sensorial quality of teff injera. Accordingly there was no significant difference between hammer mill injera, disk mill injera and blade mill injera in color, odor, and taste, number of eyes, eye distribution and the top and the bottom surface of injera. The three injeras were described as white in color, acceptable in flavor, slightly sour in taste, with a non-sticky and non-powdery top and bottom surface, and with many eyes and regular eye distribution. However, a significant variation in texture strength (degree of softness), eye size, and overall acceptance were identified. Disk mill injera (6.9) has a significantly softer texture than hammer mill injera (4.10) and blade mill injera (3.5). Hammer mill injera and blade mill injera, on the other hand, had considerably higher eye size scores, 6.0 and 6.1, respectively, than disk mill injera (1.6). The majority of injera consumers are more focused on their organoleptic acceptability than on nutritional value, microbiological safety, and cleanliness (Gill *et al*., 2020).

2.11. Microbial Quality of Injera

Injera spoils due to the growth of yeast, mould, and other microorganisms after baking, which imparts an undesirable flavour and colour to the injera and renders it unfit for consumption (Rawat, 2015). Microorganisms are thus the most prominent cause of food deterioration, particularly in injera, due to their activity and proliferation. The types and numbers of microorganisms found in food are mostly determined by the type of food and the degree of contamination (King, 2009). As a result, food preservation is primarily associated with the prevention or reduction of microbial activity.

The teff flour, water, and vessel are all important sources of inoculum for teff fermentation. Teff seeds are contaminated with a wide range of soil and fecal particles during the traditional threshing process (Desiye *et al*., 2017). According to this study the microbiological alterations that occur during the traditional fermentation of teff batter made from "Kuncho" and "Magna" flour have been investigated. The total aerobic mesophilic counts were high (cfu/mL) and increased consistently until 48 hours of injera batter fermentation, with the LAB and yeasts being the main microorganisms.

The aerobic mesophilic counts of teff injera ranged between 1.83 to 2.39 log10 $cfug^{-1}$ and the yeast and mold counts ranged between 0.39 and 2.35 $cfug^{-1}$ that are stored at different storage days (Godebo *et al*., 2019). On the other hand the total aerobic bacteria plate count and total yeast-mold count of teff injera on the day of baking were not detected. But on the day three and six storage 3.88 and 7.53 log $cfug^{-1}$ for total aerobic bacteria plate counts while the total yeast-mold count is 3.87 and 7.53 on day 3 and 6 storage respectively (Anberbir *et al*., 2023).

2.12. Nutritional Composition of Teff Injera

Teff injera was found to have better nutritional value than injera made from other cereal grains (Mihrete & Bultosa, 2017). According to this study the proximate composition of teff injera ranged from 11.08-12.66% (protein), 2.15-2.53% (fat), 1.35-1.45% (fiber), 60.74-69.60% (CHO) and 348.07-337 kcal/100g (energy). Woldemariam *et al*. (2019) reported proximate composition of teff injera as 5.82% moisture, 3.51% fiber, 10.62%b protein, 1.02% fat, 3.2% ash, and 79.34% carbohydrate and 374.81kcal/100 g energy. In addition to supplying proteins and calories, teff injera has a high nutritional value that includes significantly superior amino acid composition, especially lysine, which is limited in other cereal-based injera (Baye, 2014).

The mineral and ant-nutritional contents of teff injera were reported by Yegrem et al., (2021). Accordingly teff injera contains 167.69 mg/100 g calcium, 15.43 mg/100g iron, 2.40 mg/100g zinc, 12.12 mg/100 g tannin and 122.54 mg/100g phytic acid. The mineral and phytochemical content of teff injera was reported by Anberbir et al., (2023) as 12.82mg/100g iron, 136.22mg/100 g calcium, 1.14 mg/100 g zinc, 5.90mg catechin equiv. /100g tannin, 128 mg/100 g phytic acid and 132.42 GAE/100g total phenolic compounds.

3. MATERIALS AND METHODS

3.1. Experimental Materials and Sample Preparation

Tseday variety of teff grain which is approved by Amhara National Regional State Seed Quality and Quarantine Authority was collected from Adet Agricultural research center. A total of thirty five (35) kilograms of teff sample was collected for both preliminary and main research work. The variety was selected based on its popularity, resistant to short rainfall, high yield and early maturity in Ethiopia(Korji, 2018, Tadesse *et al*., 2016). The raw teff was pre basic seed of 2014 E.C. meher season grain and the moisture content when packed by 21/08/2014 E.C was 9±0.1% on dry basis. Then teff grain was manually cleaned by winnowing and sifting to remove foreign materials and debris such as stone, soil, straw and any non-grain materials followed by sun dried before milling. The cleaned and sifted sample was milled into different particle size using local attrition miller (stainless steel pin miller (china)) which is commonly used for commercial teff grinding in Ethiopia. The flour was subsequently separated into different particle-size ranges using different mesh size of sieve.
The flour particle size was evaluated by passing the teff flour through an automatic standard sieve (Y. Assefa *et al.*, 2018). Necessary preventive and precautionary measures were taken during sample preparations to avoid adventitious contamination by wearing gloves, using clean plastic polyethylene bags and keep sample preparation environment clean. The flour prepared was packed with polyethylene plastic bag and kept at refrigeration temperature (4°C) until product formulation and further functional and physico-chemical analysis.

3.2. Study Site

The experiment was carried out in Bahir Dar Institute of Technology (BiT) Food Engineering laboratories located in Bahir Dar, Ethiopia at which the city lies between the latitude of 11^{o}57'00"N and longitude of 37^{o}36'00"E with average altitude of 1800m above sea level (Weldegerima *et al.,* 2018). Analysis of phytate content of both flour and injera was done at Addis Ababa University College of Natural and Computational Science. The pasting and rheological properties of flour were determined in Addis Ababa Science and Technology University. The mineral contents of samples were evaluated in Bahir Dar University Peda Campus department of chemistry.

3.3. Experimental Design

The study was laid out in completely randomized design (CRD) with full factorial arrangements with two ways analysis of variance performed in triplicate. The experiment was performed with two factors comprising; flour particle size and dough kneading time. Maximum and minimum levels of factors were investigated by doing a preliminary analysis. Experimental design of the research was with specific value of 4 minutes, 8 minutes, and 12 minutes dough kneading time with a constant moderate kneading speed of #K_2 (164rpm) and fine (0-180µm), medium (181-355µm) and coarse (356-500µm) flour particle size. Whole teff flour commonly used by society to make injera was taken as control flour which holds all flour particle size including 0.81% left on 500µm sieve size.

Table 3.1. Experimental design of the research/treatment combination

Flour Particle size(Factor 1)	Dough Kneading Time (Factor 2)		
	KT_1	KT_2	KT_3
FFPs	FFPs*KT_1	FFPs*KT_2	FFPs*KT_3
MFPs	MFPs*KT_1	MFPs*KT_2	MFPs*KT_3
HFPs	HFPs*KT_1	HFPs*KT_2	HFPs*KT_3
CF	CF*KT_1	CF*KT_2	CF*KT_3

Where:- KT=Kneading time, FPs= Flour Particle size and K_1, K_2, K_3 are 4 min,8 min,12 minutes respectively and FFPs, MFPs, HFPs are fine (0-180µm), medium (181-355µm), coarse flour particle size (356-500µm) respectively and CF= control flour

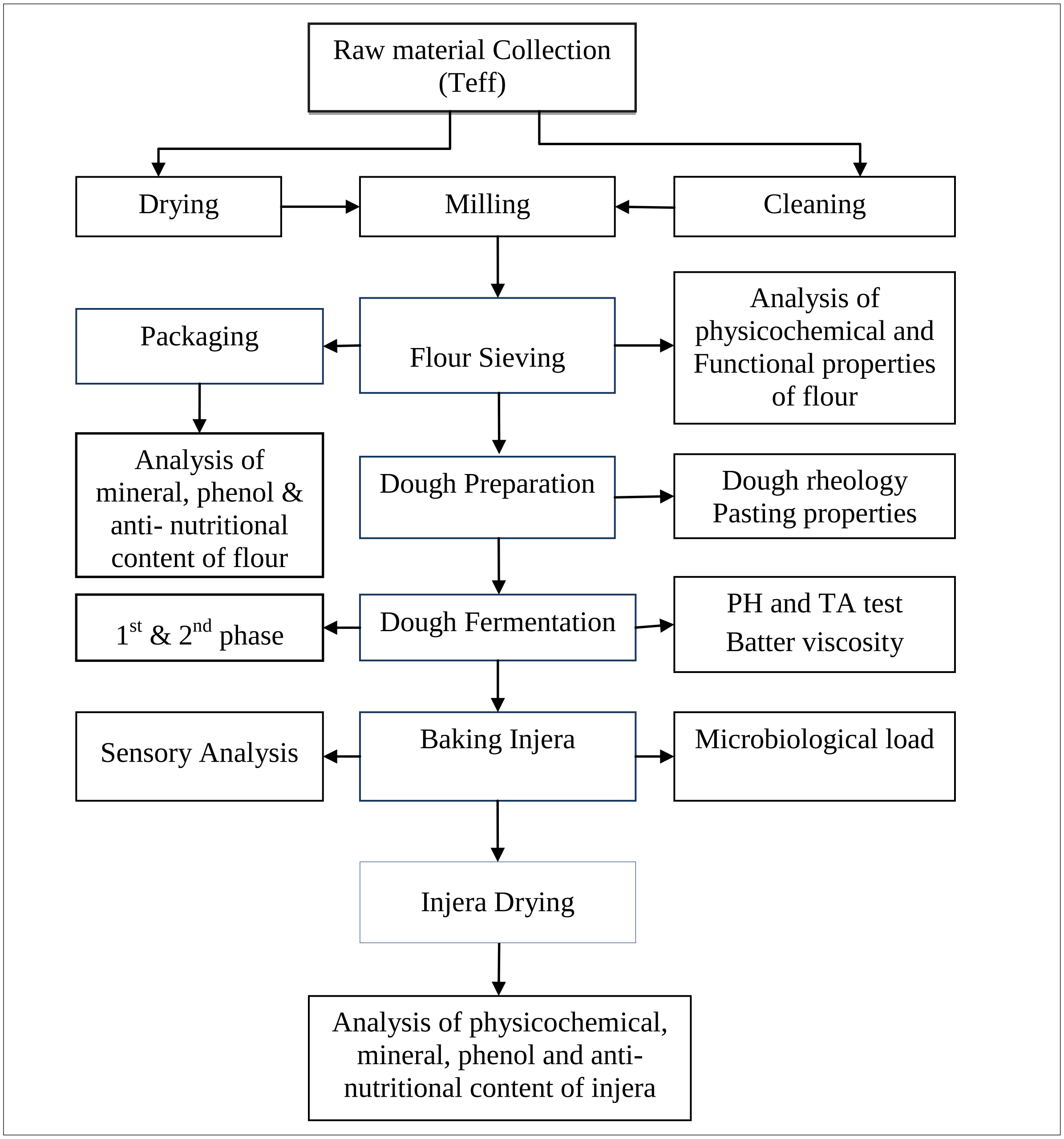


Figure 3.1. The overall experimental framework of the research

3.4. Determination of Flour physical Properties

3.4.1. Flour Particle Size Determination

The flour particle size was evaluated by passing through an automatic standard sieve (SHANG HAI HONGZHOU, China). The particle size of teff flour was conducted by sieve shaker as stated in AACC based on milling standard of grain sieves. The sieve openings of 500µm, 355µm and 180µm were used for experimental samples (Y. Assefa *et al*., 2018). The flour fractions passing through 180µm were named as fine flour, flour fractions passing through 355µm and retained on 180µm was named as medium flour particle size, flour fractions passing through 500µm and retained on 355µm was named as coarser flour particle size. The commercial whole teff flour was used as control flour. The percentage fraction of the sample obtained with each sieve was measured by weighing (Abebaw, 2020). The weight rating of each size range was calculated as equation below

% Weight of obtained flour= $\frac{\text{weight passed through sieve}}{\text{Total weight}}\times 100$……………..equation (3.1)

3.4.2. Determination of Flour Moisture Content

Flour moisture content was determined according to AOAC (2005) method 925.10 (Nielsen, 2010). A moisture dish was washed and dried in an oven (model PF120 (200) England) at 105^{o}C for 1 hour and placed in desiccator to cool. The weight (Using Precision digital balance) of the empty moisture dish (W1) was recorded. Sample (5gram) was weighed in dry moisture dish (W2) and kept in an oven set at 105ºC for 6hour until constant weight reached (W3). The moisture content of flour was then determined as:

Moisture content (MC %) = $\frac{W2\text{-}W3}{W2\text{-}W1}$ x100…………………………………..Equation (3.2)

Where: W1 is mass of the empty dish (g), W2 is an initial mass of the sample and dish before drying (g) and W3 is mass of sample and dish after drying (g)

3.4.3. Flour Color

The color of the teff flour samples were evaluated according to the method used by Abebe et al (2015) using spectrophotometer (color spectrophotometer CM 600d, Konica Minolta, Inc, Japan). The results were obtained by the coordinates of CIE L*a*b. White standard tile was used for calibration as standard reference reading (L*, a*, b*). The values of the colors are expressed as L* (whiteness or darkness), a* (redness or greenness), and b* (yellowness or blueness). The flour samples were placed on the colorless Petri dish and measured. The hue (h) and the Chroma (C*) were calculated from equation 3.3 and equation 3.4 respectively as used by Y. L. Assefa et al (2018).

$h = \tan^{-1}(b^*/a^*)$ …………………………………….…………..……... equation 3.3

$C= ((a^*)^2 + (b^*)^2)^{1/2}$……………………………………………………… equation 3.4

Where: Hue angle is the color perceived by the naked eye and the color measured in degree and Chroma is the chroma city coordinate which is perpendicular to the distance from lightness.

3.4.4. Flour Water Activity Analysis

The water activity of flour samples were determined using Aqua Lab Life water activity measuring unit manufactured by Decagon a_w meter, (2004 in USA). Each sample was half filled in a small plastic cup supplied with the instrument and inserted in to the instrument. The water activity of each sample was recorded from an automatic display (Carter *et al.*, 2015).

3.4.5. Bulk Density

Bulk density of the flour samples were measured by funnel method according to the method used by H. W. Woldemariam et al., (2021). The flour sample was filled into the measuring cylinder (100mL volume) using conventional funnel. The top layer of the powder was carefully flattened with a strip of iron. A specified quantity of the flour sample was transferred into an already weighed measuring cylinder ($W1$). The bulk density was calculated as weight of sample per unit volume of sample (g/mL) (Liu *et al.*, 2013).

3.4.6. Flour Pasting Properties

The Pasting properties of flour samples were analyzed by Rapid Visco Analyzer (RVA) as the AACC method 76–21.02 adopted by Alemneh et al., (2022). Flour suspensions were used to record flour viscosity profiles (8%; 28 g total weight). Flour (3.5 g) was weighed into a dried empty canister, and 25 ml of distilled water was dispensed into the sample canister. The mixture was fully mixed, and the canister was inserted into the RVA according to the manufacturer's specifications. The suspension was held at 50°C for 1 minute, heated at a uniform rate to 95°C for 8 minutes, then held at 95°C for 5 minutes before cooling to 50°C within 8 minutes, and finally held at 50°C for 1 minute. With the use of Thermocline for Windows Software linked to a computer, the pasting parameters registered during analysis in RVA include peak viscosity (PV), viscosity at trough (TV), and final viscosity (FV) were recorded, and breakdown (BDV, which is PV minus TV) and setback (SB, which is FV minus TV) were determined. The results were expressed as centipoise Unit (cP) for all parameters except pasting temperature and pasting time, which is expressed in ^{o}C and minutes respectively.

3.5. Functional Properties of Flour

3.5.1. Water Absorption Capacity

Water absorption capacity of different flour particle size was determined by the method of centrifugation as used by Yu et al (2007). About 2.5 grams of flour sample was weighed into the clean and dried centrifuge tube (W1) and (25mL) of distilled water was added. The tube containing sample and distilled water was gently shacked by vortex mixer for 10 minute. The samples were allowed to stand for 30 minute at ambient temperature and transferred to centrifuge for 10 minutes at 3300rpm. Water was decanted by inverting the tube. The weight of the sediment with the tube was taken (W2). Finally the decanted water was dried in dry oven for 6hr at 105^{o}C and added to sediment as dry sample (W3). Water absorption capacity was calculated as in equation 3.5.

WAC (ml/g) = (W3-W2)/W1……………………………………………equation 3.5

Where: W3= is weight of empty tube + Sample after centrifuged and decanted
W2= is weight of empty tube + sample before centrifuged

W1= is weight of dry sample

3.5.2. Water Solubility Index (WSI) and Swelling Power (SP)

Water solubility index and swelling power of flour was estimated by the method used by Awolu et al (2015). About 2.5 g of sample was weighed in to 100mL conical flask hydrated with 50 mL of distilled water and shaken for 10 minutes with vortex mixer. The conical flasks with its contents were put in a water bath maintained for 85°C for 40 minutes with stirred regularly. After heating, the resulting slurry was cooled to room temperature and was quantitatively transferred into centrifuge tube by washing with 15ml distilled water and centrifuged at 3300rpm for 10 min. The supernatant was decanted into a pre-weighed evaporating dish, dried in an oven at 105°C for 6hr. to a constant weight for estimation of WSI of the product. The dried supernatant and residue/ sediment were weighed. The swelling power and water solubility index was calculated as follows:

$$\%WSI=\frac{\text{Weight of soluble or dry supernatant}}{\text{Weight of sample}}\times 100 \quad \text{……………….Equation (3.6)}$$

SP (g/g) = Weight of sediment/ (weight of dry sample-weight of dry supernatant…………………………………….…………....Equation (3.7)

3.5.3. Oil Absorption Capacity

The procedure used for water absorption capacity was used for oil absorption capacity determination except that oil was used instead of distilled water. About 2.5 g of flour sample (W1) was weighed into pre-weighed 50 mL centrifuge tube and thoroughly mixed with 25mL (initial oil volume) of refined soy bean oil using vortex mixer. The sample-oil mixture was centrifuged at 3300 rpm for 10 minutes. Immediately after centrifugation, the supernatant was carefully poured into a graduated cylinder, and the volume was recorded (unabsorbed oil volume) (Karki, 2016). The volume of the unabsorbed oil was determined and OAC was calculated as follows:

$$OAC=\frac{\text{Weight of Oil Absorbed}}{\text{Sample Weight}} \quad \text{…………………………………….... (Equation 3.8)}$$

3.5.4. Water Absorption Index (WAI)

Water absorption index (WAI) (g/g) was determined by the method outlined by Yousf et al., (2017). The volume that the granule or starch molecule holds after swelling due to an excess of water is measured by the water absorption index (WAI). Flour sample of about 2.5 g was suspended in distilled water at room temperature for 30 minutes, gently stirred during this period and then centrifuge at 3300 rpm for 10minutes. The supernatant liquid was poured carefully in to tared evaporating dish. The remaining gel was weighed and WAI was calculated as grams of gel obtained per gram of solid.

$$\text{Water absorption index (WAI) (g/g)} = \frac{\text{weight of sediment}}{\text{Weight of dry solid}} \quad \text{……………..……Equation (3.9)}$$

3.6. Preparation of Dough and Injera

3.6.1. Dough Preparation

Teff dough was prepared according to Baye et al., (2013) and Yetneberk et al., (2004) with little modification on kneading time and ratio of water and flour. Amount of starter (*Ersho*) equal to 5% (50mL) of flour weight base was initially added to each sample of teff flour dough. This starter was prepared three days before dough preparation started for initiation of dough fermentation. The teff flour of 1kg was mixed with 1.5L potable water and mixing for the dough kneading times (4, 8 and 12 min) by constant kneading speed of #K_2/164 rpm. The dough was pressed down to the bottom of the bowl to allow the fermentation temperature of the bowl. The water left back from dough preparation was added over the pressed dough to protect the surface of the dough from molding during fermentation. Then the dough was stored for 60 hours at room temperature for the first phase fermentation.

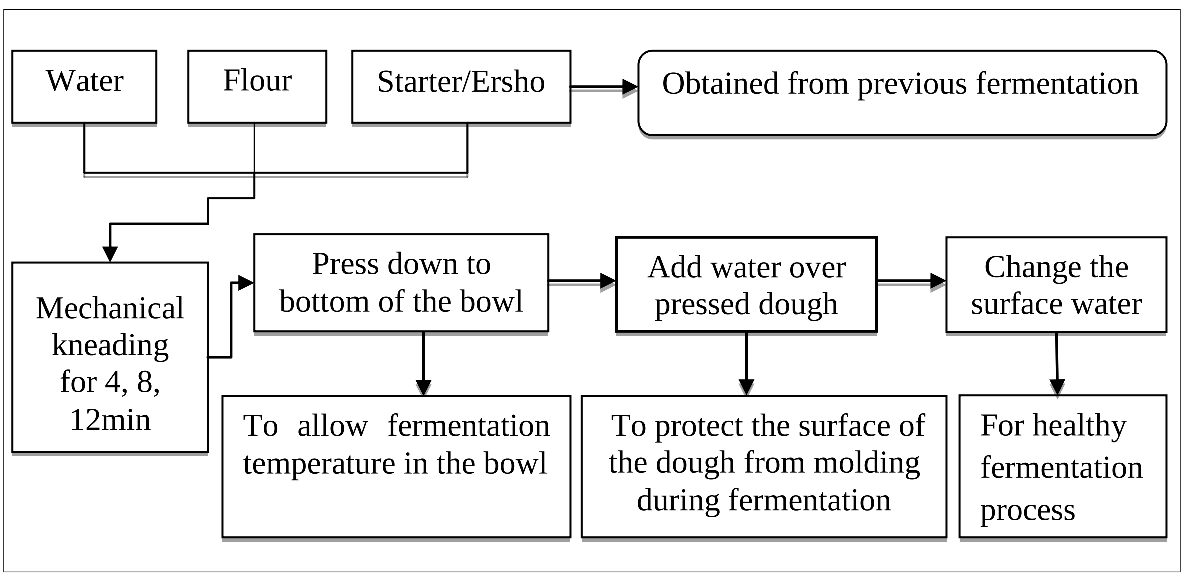


Figure 3.2. Flow diagram of dough preparation

3.6.2. Determination of Batter Viscosity

The viscosity of the batter was evaluated by a rotational digital viscometer (Visco Star plus FUNGI LAB S.A.) with R4 spindle number. The instrument was leveled with its appropriate spindle number (R4) and rpm needed to evaluate were all set to its standard. The selected measuring feature was displayed on the LCD of the instrument and about 50 mL of ready to bake batter was brought to the Viscometer and loaded spindle was immersed up to the mark into the beaker containing the sample. The viscosity of the batter was measured just before baking for all the samples by controlling the temperature using water bath at 30^{o}C *(*Xu *et al*., 2014)

3.6.3. Batter pH Test

Dough prepared from different particle size with different kneading time was fermented and thinned to batter dough ready to bake injera. The pH of fermented dough and thinned to batter (ready to bake) was measured by scientific electronic bench top pH meter (Model: PHS-25/3C) after calibrating with buffers at pH 4.0 and 7.0. The pH electrode was immersed in the beaker containing the batter samples (fermented), and a stable result was recorded.

3.6.4. Determination of Titratable Acidity of Batter

Titratable acidity (TA) of batter was evaluated by 0.1N of NaOH in the presence of phenolphthalein as color indicator. 0.1N of sodium hydroxide solution and phenolphthalein indicator were prepared for the measurement of TA of the batter. Using burette titrate, ten (10) mL of the sample was combined with 100 mL of distilled water and titrated with 0.1 N NaOH to an endpoint PH of 8.2. (Titroline Alpha Plus TA 20, S.I. GmbH, Mainz, Germany) in the presence of three drops of phenolphthalein indicator. Each titration's volume of NaOH was recorded when the color changes to pink, and the titratable acidity was expressed as percent lactic acid (AOAC, 1995) using the Equation 3.10 below.

$$\%\ \text{Lactic acid} = \frac{(\text{mL of NaOH used})\times(0.1\text{N NaOH})\times 0.09\times 100}{\text{Volume of of sample}} \quad \text{..................Equation 3.10}$$

3.7. Dough Rheological Properties

The dough samples were subjected to dynamic oscillatory rheometer using a Kinexus Knx5210 (United Kingdom) rheometer with a serrated surface parallel plate (40 mm diameter) and with a 1mm gap at 25^{o}C, in accordance with the methodology given by (Abebe & Ronda, 2014). Samples were given five minutes to relax before beginning the experiment. The frequency of 10 to 0.1Hz sweeps were conducted in the previously identified linear viscoelastic zone. At a frequency of 1 Hz, stress sweeps were performed from 0.01 to 500 Pa. The dough was made three times, and the measurements were done in triplicate. The elastic modulus (G', Pa), viscous modulus (G", Pa), and tan δ (G"/G') were measured for a stress value inside the linear viscoelastic region using a frequency sweep test (w, Hz). The coefficients G', G", and (tan δ) represent the elastic, viscous, and loss tangents at a frequency of 1 Hz, respectively. The frequency ranges (1–10 Hz) were used for fittings, and a linear double logarithm curve was carefully obtained. The loss tangent was calculated from viscus modulus and elastic modulus as equation 3.11 below.

$$\tan \delta = \frac{G''}{G'} \qquad \text{equation 3.11}$$

3.8. Injera Preparation

The preparation of injera was performed as stated by Gebru et al., (2019). About 1Kg of teff flour was mixed with 1.5L of potable water and the dough was fermented at room temperature for 60 hours with adding starter culture (Ersho). After 60 hour of first phase fermentation which gives the injera its tangy taste. The surface water formed on top of dough was discarded. About 10% of the fermented dough was thinned with 750 mL water and boiled for 10 minutes to gelatinize the starch. Gelatinization (cooking) was used to achieve two goals: first, to make the dough more cohesive (dough binder) and second, to give easily fermentable carbohydrate to leaven the injera. The gelatinized batter, often known as 'absit,' was cooled to room temperature and then reintroduced to the fermenting dough. Water (200 mL) was added after thorough mixing to thin and form the right consistency of batter and the batter was fermented at room temperature for 6hours. Then the fermented batter was putted in a circular pattern on a heated clay griddle (metad) with a diameter of 50 cm, covered and baked for about 3 minutes on 220^{o}C (Girma *et al*., 2013b).

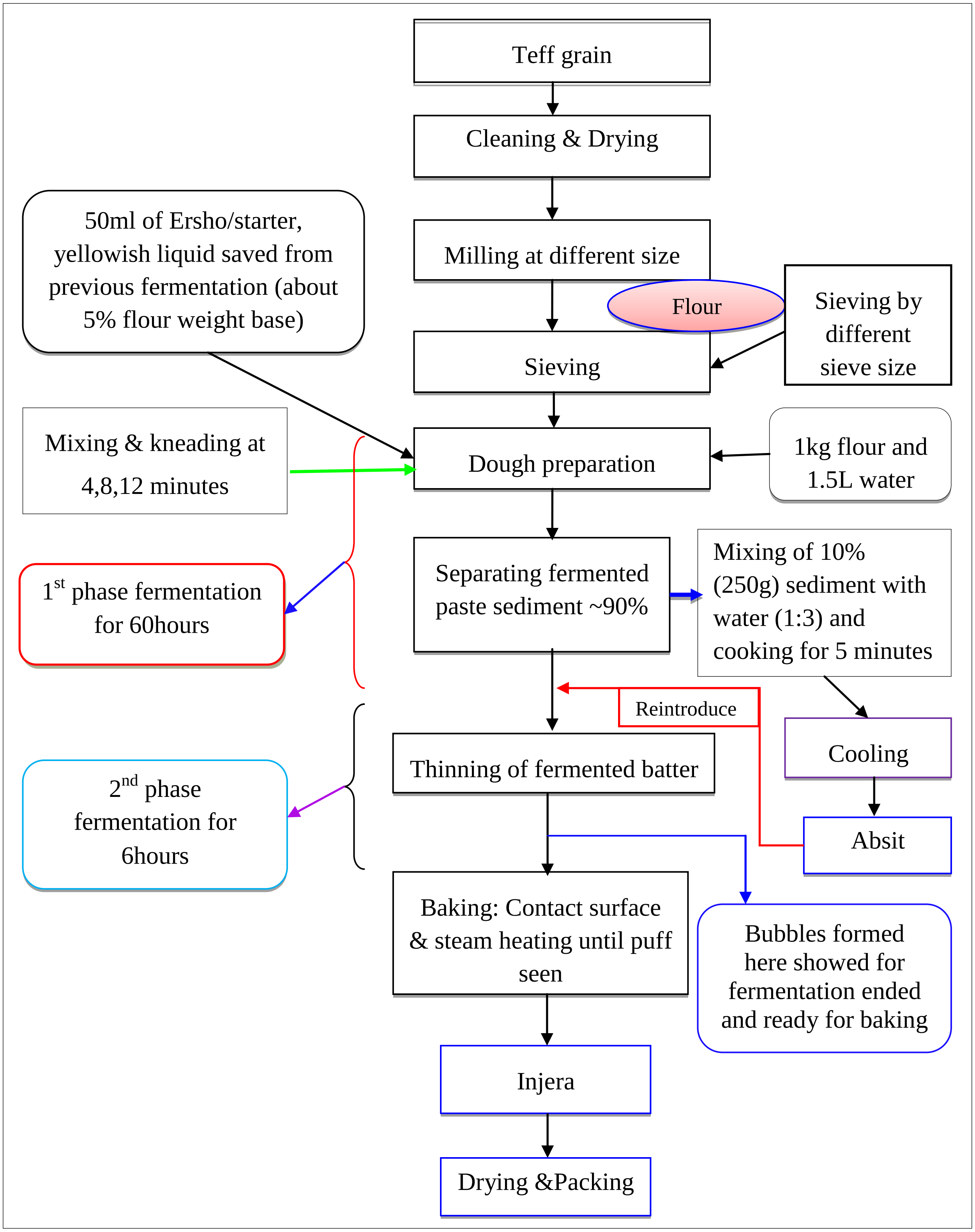


Figure 3.3. Procedural injera preparation for research work

3.9. Physico-Chemical Analysis of Injera

3.9.1. Color Analysis

The color of injera was evaluated according to the method used by Abebe et al (2015) using spectrophotometer (color spectrophotometer CM 600 D, Konica Minolta, Inc, Japan). The results were obtained by the coordinates of CIE L*a*b. White standard tile was used for calibration as standard reference reading (L*, a*, b*). The values of the colors are expressed as L* (whiteness or darkness), a* (redness or greenness), and b* (yellowness or blueness). The injera samples were placed on the colorless Petri dish and measured. The hue (h) and the Chroma (C*) were calculated from equation 3.3 and equation 3.4 respectively as used Y. L. Assefa et al (2018).

$h = \tan^{-1}(b^*/a^*)$……………………………………….…….…….………. equation 3.12

$C= ((a^*)^2 + (b^*)^2)^{1/2}$……………………………………………………….. equation 3.13

Where: Hue angle is the color perceived by the naked eye and the color measured in degree and Chroma is the chroma city coordinate which is perpendicular to the distance from lightness.

3.9.2. Instrumental Texture Analysis

Using a texture analyzer (TA-XT plus texture analyzer (Stable Micro Systems, Godalming, UK) similar to that developed by Fox et al., (2020) the texture/firmness of injera was assessed. Prepared injera was sliced into uniform strips by using clean and sharp knife for texture analyzer. The injera pieces were kept at room temperature in polyethylene bags for 1st, 2nd and 3rd days of storage. The peak force of stored injera was measured by using a texture analyzer in compression mode with a sharp blade cutting probe and TA 90 heavy-duty platform. The following testing profiles were used to measure the thickness and firmness of injera slices using calipers. Pre-test speed (1.0mm/s), test speed (3.0mm/s), post-test speed (10.0mm/s), distance (15mm) and trigger type (0.049N auto). The sample pieces were positioned over the bending rig's vertical struts, which were spaced 30mm apart, and clamped at both ends. Over a 15mm distance, the strips were squeezed at a constant rate of 10mm/s. The hardness cutting of the bending force was used to compute the peak force (N) of each injera prepared from different flour particle size and varied dough kneading time.

3.10. Sensory Evaluation

Prepared injera from different flour particle size and dough kneading time was evaluated for the sensory parameters by the untrained panel of 50 (25 male and 25 female) judges based on the five point hedonic scales. Panelists were randomly selected from students and staffs of Bahir Dar Institute of Technology who were willing to take part in the sensory evaluation activity and regularly consume injera as their staple food (Stone & Sidel, 2004). Eight injera quality descriptors were assigned for the sensory evaluation. The injera quality descriptors used in sensory evaluations were color, taste, eye size, rollability, texture, eye distribution, and top and bottom surfaces of injera and overall acceptability of injera. Each factor's relative importance was compared numerically on a scale of 1–5, ranging from dislike very much to like very much. Samples were coded and arranged in a random order and placed in identical containers and served to the sensory panelists to prevent any prejudice. A glass of potable water was provided for panelists so that they rinse their mouths between samples (Olaoye *et al*., 2007). Panelists were instructed for the terms in the quality descriptors and ways of evaluating for those who were new for sensory analysis yet. Using a consumer-focused sensory panel, the impact of dough kneading duration and teff flour particle size on the sensory qualities of injera was evaluated. Sensory evaluation was performed three hours after baking of injera (Y. Assefa *et al*., 2018).

3.11. Microbial Analysis of Injera

Fresh baked injera was taken aseptically from every quarter parts of injera and blended for microbial load analysis. AOAC, (2005) technique was used to count total bacteria and mold-yeast, on injera samples after 1^{st}, 3^{rd}, and 5^{th} days of room temperature storage. The samples of fresh injera was packed and stored separately to analyze microbial load. All materials were sterilized during injera taking and media preparation. Plate Count Agar (PCA) was used as a media for total microbial load analysis and Potato dextrose agar (PDA) as mold and yeast growth media.

3.11.1. Analysis of Total Bacterial Load

Plate count agar (standard methods agar) was prepared according to the manufacturer`s direction written on the package of the media as the method used by Anberbir et al., (2023).

About 23.5 grams was suspended in 1000mL of distilled water and heated to the boiling to dissolve it completely. Then the boiled media was sterilized by autoclaving at 121°C for 15 minutes (15lbs pressure). It was cooled to 65^{o}C in water bath before used. About 1gram of peptone and 8.5 grams of NaCl was mixed to 1000mL of distilled water to prepare peptone water and sterilized at 121°C for 15 minutes. About 10 grams of Injera samples were collected aseptically and homogenized in 90mL sterile 0.1% peptone water in a blender for 2 minutes, followed by serial dilutions. One milliliter of each dilution was pour plated in sterile Petri dishes, the stomacher dilution represents the 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilution prepared by using 9ml peptone water for the 1st day storage analysis and 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} , $10^{-8}$and 10^{-9} for the 3rd and 5th day storage. About 20 mL of the prepared PCA was poured on the petri dish aseptically and allowed to solidify at room temperature. Serial dilutions of the suspension were prepared and about 1mL aliquots were taken using micropipette and spread on the solidified PCA and incubated at 30°C for 72 hrs. The counts of visible colonies were made by using colony counter and expressed log10 cfu/g of samples before statistical analysis (Achenef *et al*., 2013). On plates with 25 to 250 colonies of total aerobic bacteria were counted using a colony counter as cfu/g.

3.11.2. Analysis of Yeast and Mold Load

Based on the method used by Godebo et al., (2019), potato dextrose agar was prepared according to the manufacturer`s direction written on the package of the media. About 39 grams was suspended in 1000mL of distilled water and boiled to dissolve it completely. Then the boiled media was sterilized by autoclaving at 121°C for 15 minutes (15lbs pressure). About 1gram of peptone and 8.5 grams of NaCl was mixed to 1000mL of distilled water to prepare peptone water and sterilized at 121°C for 15 minutes. About 10% of tartaric acid was used to acidify the PDA media. Tartaric acid (10mL) was used for 1000mL media. Injera samples (10 grams) was collected aseptically and homogenized in 90mL sterile peptone water 0.1 percent in a blender for 2 minutes, followed by serial dilutions. One milliliter of each dilution will be poured into sterile Petri dishes, with the stomacher dilution representing the 10^{-1} dilution, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} dilutions prepared by using 9ml peptone water tubes and potato dextrose agar (PDA) with chloramphenicol addition and stored at room temperature for 5 days. Using a colony counter, counts of visible colonies were made and expressed as log CFU/g. Finally the numbers of colony forming units (CFU) per ml of original stock were calculated by the equation 3.14.

The colonies of 15 to 150 yeast-mold count were taken into consideration for the appropriate yeast mold load on injera.

$$CFU/mL = \frac{\text{No of colomies} \times \text{Total dilution factor}}{\text{Volume of culture plated in mL}}$$ ....Equation 3.14

3.12. Drying of Injera Samples

In an air oven drier (Model: DHG- 9140A; Zenith Lab Inc, China), the freshly baked injera was dried at $70^{o}C$ for 6hours by spreading over aluminum foil and grinding into a fine flour and the packed samples were stored at $4^{o}C$ for further experimental analysis (Girma *et al.*, 2013a).

3.13. Analysis of Proximate Composition of Injera

3.13.1. Moisture Content

The moisture content of formulated injera from different particle size and different kneading time was determined according to AOAC (2005) official method 925.10. Moisture dish was washed and dried in Oven at temperature of $105^{o}C$ for 1hour and placed in desiccator for cooling. The weight (Using Precision digital balance) of the empty moisture dish (W1) was recorded. Almost 5gram sample was weighed in dry moisture dish (W2) placed in an oven set at 105ºC for 6hour until constant weight reached (W3). The moisture content of injera sample was then determined as follows:

$$\text{Moisture content (MC \%)} = \frac{W2-W3}{W2-W1} * 100$$ ....Equation 3.15

Where: W1 is mass of the empty dish (g), W2 is an initial mass of the sample and dish before drying (g) and W3 is mass of sample and dish after drying (g)

3.13.2. Crude Protein Content

The crude protein content of the injera sample was determined by micro-Kjeldahl method as stated in the AOAC (2005) Method 991-20 (Kjeltec 8400, Auto Sample Systems, Foss, Sweden). About 0.5 g flour sample was weighed into Kjeldahl digestion flasks and 2 grams of catalyst mixture ($CuSO_4$ and $NaSO_4$ in the ratio of 1:10) was added in to each flask. Then, 6 mL of concentrated H_2SO_4 (98%) was added and the sample was digested for 3 hours at a temperature of 420°C until the solution was clear white. With the completion of the digestion (when the digested sample becomes colorless or light blue) the samples were allowed to cool. After the samples were cooled, 50 mL of distilled water was added into each digestion flask followed by 40 mL of 40% NaOH. Immediately the contents were distilled by inserting the digestion tube line into the receiver flasks that contain 25 mL of 4% boric acid solution. The collected ammonia distillate was then titrated against a standardized 0.1N HCl until the end of the titration is attained (where the titration color changes from blue to pink). Then the volume of HCl consumed to reach the titration end point was read from the burette and the %nitrogen content was calculated as follows:

$$\text{Nitrogen}(\%) = \frac{(V_{HCl} - V_{HCl\ blank}) \times N_{HCl} \times 14.00}{\text{sample weight in dry matter basis}} \times 100$$

Where V_{HCl} is volume of HCl in litter consumed to the end point of titration, $V_{HCl\ blank}$ is the volume of HCl in litter consumed to titrate the blank, N_{HCl} is normality of HCl used and 14.00 is the molecular weight of nitrogen. Percent nitrogen was expressed on dry matter basis and the resulting value multiplied by a factor of 6.25 to obtain percent protein content of each variety at each location. Analytical grade urea was used as a control.

3.13.3. Crude Fat

Fat content of injera and flour samples were evaluated by using semi-continuous solvent extraction method (Soxhlet method) according to AOAC (2003.05) method 920.39. About five (5) gram of sample was weighed and transferred to thimble. Then the thimble containing sample was placed into extraction chamber. 150 mL of n-hexane was added and power turned on with the continuous water condenser released. After six (6) hour extraction, power turned off and extracted fat containing little amount of hexane was transferred from the extraction flask into a pre-weighted small beaker. The chamber was rinsed with hexane to collect the left fat. The hexane transferred to beaker with extracted fat was evaporated for 1 hour in drying oven of 105°C. Then it was removed from the oven and cooled in desiccator. The beaker and final crude fat content were re-weighed again. The Crude fat content was then computed as the equation 3.18

Crude fat (%) = $\frac{W2\text{-}W1}{\text{Weight of sample}} \times 100$ ……………………….Equation 3.18

Where W1 = Weight of the empty beaker in gram (g)

W2 = Weight of the beaker and the dried crude fat in gram (g)

3.13.4. Total Ash Content

Total ash content of samples was analyzed according to, AOAC (2005) method 923.03 using the combustion method in a muffle furnace at 550^{o}C. The crucibles were washed and dried in drying oven for 2 hour at 105^{o}C. Clean and dried crucibles were weighed to the closest milligram after cooling in desiccators (with granular silica gel) for roughly 30 minutes at room temperature (W1). About 5 g of samples (in triplicate) were placed in the pre-weighed crucibles and weighed (W2). Then the sample was thoroughly charred in a fume hood by placing it on a hot plate, increasing the temperature slowly until smoking ceases. After the completion of charring, the sample was placed in muffle furnace at 550^{o}C for 5 hours. The ignition was continued by cooling for 1hour and weighing until getting a clean and white ash to the nearest milligram (W3).

The ash content was analyzed by calculating using Equation 3.19

Total ash content (%) = $\frac{W3\text{- }W1}{W2\text{-}W1} \times 100$………………….Equation 3.19

Where

W1 = Mass of the Crucible (g)

W2 = Mass of the Crucible and the Sample (g)

W3 = Mass of the Crucible and Dried Sample (g)

(W2-W1) = Initial Sample Weight (g)

(W3-W1) = Weight of Ash in (g)

3.13.5. Crude Fiber Content

Crude fiber content of teff flour and injera samples were determined by AOAC 978.10 (ISO 6865:2000) Weende method. Acid, alkali and acetone solution was prepared to wash the ingredients in the sample to differentiate fiber and mineral from other proximate content or organic matter (Yegrem *et al.*, 2019). Two (2) grams of samples was weighed and transferred into capsule. The capsule containing the sample was placed in the 600 ml beaker and 200mL of 1.25% sulphuric acid solution was added. The beaker was putted hot plate to boil and wash the sample with sulphuric acid for 30 minutes by shaking periodically. At the end of digestion with boiled sulphuric acid solution; the beaker was removed from hot plate and rest for one minute. Then the samples were washed with distilled water to remove acid residues and neutralize the pH. After 30 minutes of heating by gently keeping the level constant with distilled water, 200mL of 1.25% NaOH solution was added into beaker and again allowed to boil gently for another 30 minutes and filtering to remove proteins, some hemicellulose and lignin. Then the washing step was performed again in order to remove the alkali residues with distilled water and neutralize the pH. The samples was then washed and filtered with 1% of HCl to clear left starch and sugar and wash with distilled water. The Samples was defatted with acetone and the last washing was performed. The samples were then dried in an electric oven (Memmert 854 Schwabach, West Germany) at 130 °C ± 2 °C for 3hrs. Then, it was cooled at room temperature for 30 minutes in desiccators and weighed, finally transfers the crucibles to muffle furnace (Carbolite Aston Lane, Hope, and S20 England.) for 4 hours ashing at 550^{o}C until carbon-free and were cooled again in desiccators. Finally, the mass of each crucible was recorded.

Using the formula 3.7, the crude fiber content was analyzed.

% Crude fiber = $\left[\frac{(W3-W1)-(W4-W1)}{W2}\right] \times 100$……………………………..…….Equation 3.20

Where: W1 = weight of empty (pre-weighed) crucible, W2 = weight of initial sample,

W3 = weight of dried residue with the crucible, W4 = weight of ash with the crucible

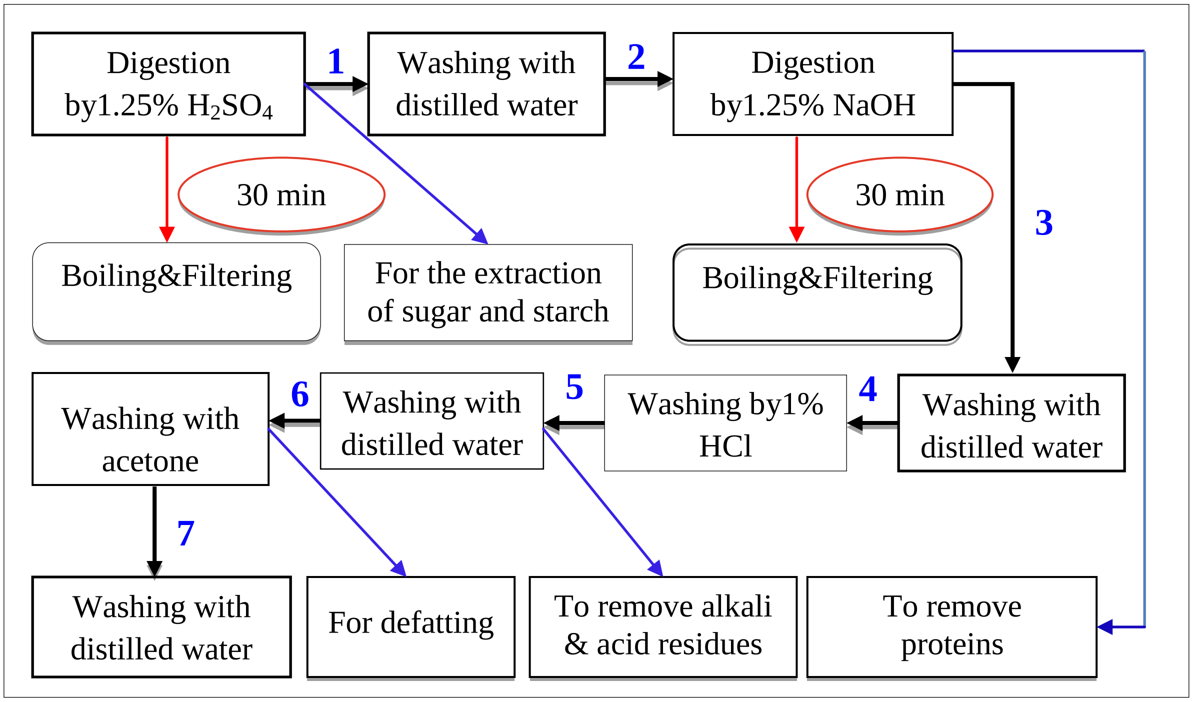


Figure 3.4. Crude fiber content determination

3.13.6. Total Carbohydrate

The total carbohydrate content was calculated by deducting the sums of the crude protein, total fat, moisture, and ash in a portion from the injera overall weight (Onyeike *et al*., 1995). The total carbohydrate amount is calculated using equation 3.3 below.

100 - (% Moisture +% Crude protein +% Crude fat +% Crude fibre + % Ash)……………equation 3.21.

3.13.7. Gross Energy

According to AOAC, (2000) the mean value of crude fat (F), total carbohydrate (CHO), and crude protein (P) were multiplied by the Atwater`s factors 9, 4, and 4 respectively, and the sum of the products was used to calculate the total energy content of the injera samples as described in the following equation..

E (kcal per 100 g) = [(9 × % crude fat) + (4 × % crude proteins) + (4 × %total carbohydrates)](AOAC, 2000; Horwitz, 2000). Equation 3.22

3.14. Analysis of Mineral Content

Atomic Absorption Spectrophotometer, the AOAC 984.27 (2005) method was used to determine iron, zinc and calcium contents of flour and injera samples. All digested tubes, extraction flask and materials used for mineral analysis was washed with soap and tap water. The materials was then soaked with 10% nitric acid for twelve (12) hours. Finally the flasks and tubes were rinsed with deionized water. Then the glassware's were dried in hot air oven drier for 90 minutes. After 90 minutes of drying the digester and flask was labelled as with sample code and blank. About 2 grams of samples were weighed and transferred to sample coded tubes. By using the tube of Italian-made Kjeldahl digester, model DK20 cleaned and dried and 20 mL of concentrated HNO_3 (65%) and 10 mL of 70% $HClO_4$ were used to digest the sample. Acid added samples were left for 12 hours at room temperature that is resulted brown color formed due to reaction of samples with HNO_3. Then the samples were heated with digester in 120^{o}C for 30 minutes. Samples were heated again for 30 minutes to get clear solution. The clear solution obtained from digestion was transferred to 100 mL volumetric flask by diluting with deionized water and filtering with Whatman No 1 filter paper. Samples were removed from filter paper with deionized water and volumetric flask was filled up to the level. At the initial a calibration curve was prepared by plotting the absorption or emission values against the standard solution in ppm with (0.04, 0.08, 0.16, and 0.32, 0.64 ppm) to calculate the values of mineral content. Standards made from stock standard solution were designed to contain the same level of acid as test solutions that had been digested. The solution was sprayed into an Atomic Absorption Spectrophotometry (PG Instruments Ltd, model AA500G, England) at 248.3 nm to get the final iron content of the sample from triplicate observations. As a result, the reading is taken from the graph, which illustrated the metal concentrations that correspond to the sample and blank absorption or emission values. 2.5 mL of lanthanum chloride was added for each sample for calcium reading. The concentrations of the samples were calculated from the absorbance values of each samples using Beer Lambert Law plot using the formula 3.23 below as used by Cherie et al., (2018).

Metal content (mg/100g) = $\left[\frac{(A\text{-}B)*V}{W}\right] \times 100$ …………Equation 3.23

Where:-

W = Weight of sample in (g)

V = Volume of extract (ml)

A = Concentration of sample solution (μg/ml)

B = Concentration of blank solution (μg/ml)

3.15. Determination of Total Phenolic Compounds and Anti-nutritional Analysis

3.15.1. Determination of Total Phenolic Compounds

The total phenolic content of the teff flour and injera samples were determined using the Folin-Ciocalteu method according to Yisak et al., (2022). Five grams (5 g) of samples were homogenized with 10 mL of 60% methanol containing 0.1% HCl, and placed in a water bath for 2 hours at 85^{o}C. After settling, the solution was transferred to a 100 mL volumetric flask, with distilled water used to fill the remaining space. The extracts were filtered using a Whatman No.1 filter paper at reduced pressure. An aliquot of 5 mL of extract was added to 15 mL distilled water, 5 mL of Folin-Ciocalteu reagent, 10 mL of saturated solution with sodium carbonate, and filling the remaining volume with distilled water. After standing for 30 min at room temperature, the absorbance was measured at 750 nm using UV spectrophotometer (Agilent, Model G6860A, Malaysia). All determinations were made in triplicate and values were calculated from calibration curves obtained with a minimum of 5 gallic acid concentrations. The linear regression was obtained between 0 and 5 mg/mL, therefore corresponding to absorbance values between 0.0 and 0.5 (R^2 = 0.9989). The total phenolic contents of samples expressed as milligrams of gallic acid equivalents per 100g (mg GAE/100g) was articulated by the following formula.

$$C\ \left(\frac{\text{mg GAE}}{100\text{g}}\right) = \frac{C1 \times V}{m} \quad \text{……………………………………………………equation 3.24}$$

Where

C = total phenolic content in mg/g, in GAE (Gallic acid equivalent);

C1 = the concentration of gallic acid established from the calibration curve in mg/ml (y = 0.1395x + 0.0041; R^2 = 0.9989);

V = the volume of extract in ml; m = the weight of extract in gram

3.15.2. Determination of Condensed Tannin

Dykes, (2019) 's (vanillin-HCl method) was used to determine condensed tannin in the teff flour and injera product. Catechin solution (1000 ppm) was firstly prepared as standard curve by using 0.24, 0.48, 0.72, 0.96 and 1.2 mL of the catechin solution that was diluted with 1.0mL methanol placed in test tubes. Test tubes containing diluted catechin were placed in water bath (30^oC) and 5 mL solution was added to first tube and 20 min timer started to count. The rest of the tubes were also filled with 5mL of vanillin solution in the 15 seconds time interval in the 20 minute of water bathing. By finishing 20 minute incubation period each test tubes were measured their absorbance at 500nm in the spectrophotometer model Cary 60 UV-Vis. Spectrophotometer was first calibrated with methanol blank. The result was used to determine the slope of the line using catechin concentrations (0.24, 0.48, 0.72, 0.96 and 1.2 mg/mL) as the x-axis and the absorbance values as the y-axis on Microsoft Excel 2010. R^2 and Slope of the Curve was determined by running a linear regression. Three HCl-methanol solutions (1%, 8% and 4%) were prepared for sample analysis. Based on the above standard 0.3 g of sample was weighed and transferred to screw cap test tube for sample analysis. About 8 mL of 1% HCl-methanol solution was added to each tube and mixed on the vortex mixer for 10 seconds, placed in water bath (30^oC) for 20 minutes. After 20 minutes incubation, test tubes were removed from water bath and mixed using vortex mixer for 10 seconds as it is recovered from the water bath. The samples were centrifuged at 4000 rpm for 10 minutes. The supernatant was decanted carefully to avoid ground sample to be transferred into the supernatant. The aliquot (1 mL) from the supernatant was taken to two different test tubes that were labelled as sample tube and blank tube. Then these test tubes were placed into water bath (30^oC) for 20 minutes. While in the water bath of 20 minutes, 5mL of vanillin reagent was added to sample tube. Vanillin reagent was prepared by mixing 8% HCl-methanol and vanillin solution that was prepared with methanol. For the blank test tubes 5mL of 4% HCl-methanol was added in the 15 seconds intervals. Finally the absorbance of the samples were measured after 20 minutes of water bath at 500 nm on spectrophotometer starting from blank and sample of each pair. The spectrophotometer was zero with a methanol blank before sample measuring. For the final determination of tannin concentration, the value of the “blank” was subtracted from that of the “sample” and calculated tannin concentration using the following equation:

Tannin concentration (mg catechin equivalent/g = $\frac{V*A/M}{Ws}$ ……………………. equation 3.25

Where

V = Volume of extract in mL (8 mL).

A = Absorbance at 500 nm (absorbance of sample minus absorbance of blank).

m = slope of the standard curve from catechin equivalent.

Ws = Weight of sample (0.3 g)

3.15.3. Determination of Phytic acid

Phytic acid content was determined using a modified version of Hang and Lantzseh rapid spectrophotometric method (Reichwald & Hatzack, 2008). Samples (0.1 g) were placed in screw-capped test tubes, and 1 mL 1M HCl was added. After that, the samples were incubated for 45 minutes in a briskly shaking water bath at 100 °C. The samples were centrifuged for 5 minutes at 13,000 × g after cooling to room temperature. Aliquots of supernatant (500 µL) was transferred to fresh tubes and diluted with 2 mL deionized water. $FeCl_3$ (800 µL) was added to the diluted solution (400 µL) or standard, and the mixture was incubated in a vigorously shaking water bath at 100°C for 45 minutes. After cooling in an ice bath for 15 minutes to allow an iron-phytates precipitate to form, the samples were centrifuged at 13000 x g for 10 minutes at 0°C. Supernatant aliquots (600 µL) were put to cuvettes, and then 800 µL of the complexing reagent was added (consisting of 1 g 2, 2-bipyridine, and 0.13 mL thioglycolic acids in 100 mL 0.2 M HCl). At 540 nm, the absorbance was finally measured. All measures were conducted in triplicate and values are calculated from calibration curves obtained with a minimum of 5 wade reagent concentrations. The phytic acids were determined using the following equation.

Phytic acid $\frac{mg}{100g} = \frac{(B\text{-}A\text{-}I)\times 10}{m\times W1\times W2}$……………………………………………..equation 3.26

Where B = absorbance of blank, A = absorbance sample, I = intercept of standard curve, m = slope of the standard curve (Y=-mx + b, R^2 = 0.994), and W1 = weight of the sample in gram and W2= weight of sample in ml(the volume of extracted from the sample (supernatant).

3.16. Statistical Analysis

The data collected from objective measurements were analyzed using analysis of variance (ANOVA) performed with procedures of the Minitab Statistical Software (MINITAB version 21). Data were compared on the basis of mean ± standard deviation (SD). Honestly significant difference (Tukey`s test) was used to separate the means when there were significant differences among treatments at a 5% level of significance. All of the parameters' fitted models were created by MINITAB during analysis and the descriptive categories were transformed to numerical scores after each analysis was completed in triplicate. Sigma plot version 12.5 and Origin Lab version 19.2b were used to create graphs.

4. RESULTS AND DISCUSSION

4.1. Physical and Functional Properties of Teff Flour

4.1.1. Flour Particle Size

The particle size of teff flour, expressed as geometric mean particle size, is a key determinant of its suitability and use in the production of high-quality injera. It has been noted that both the chemical makeup and the surface characteristics of the flour particles affect the qualities of injera (Barretto *et al.*, 2022). Grain size reduction is a crucial unit procedure for improving the surface area to volume ratio of the produced flours. Particle size distribution of teff flour is displayed in the figure 4.1.

The flour fraction that was analyzed on 180µm, 355µm and 500µm sieve size had significantly varied ($P<0.05$) that comes from the principles of milling in the attrition mill to obtain different particle size of teff flour. The percentage of 0.81% granules of teff flour retained on the top of 500µm sieve size. Most of the teff grain have the particle size range of 300-600µm (Bultosa, 2007). According to Abebaw, (2020) the different variety of teff grain retained on 710µm sieve size was from 0.76-1.93%. The raw teff fractionated to flour on milling below this particle size. About 42.54% of the teff flour particle size is mostly in the range of 181-355µm followed by 0-180µm (30.68%) and 356-500 µm (25.31%). The sieve opening (710 µm) used by the community is used as a control and 99.34% of flour was obtained. The milling intensity, mill type, moisture content and variety of grain may yield varied flour particle size (Bassi *et al*., 2021). Fine particle and control flour sizes are more distributed uniformly whereas medium and coarse particles need the incorporation of fine particle size to get its cohesiveness. Dried grain with greater mechanical milling force resulted fine particle size of flour and moisten grain with lower milling intensity obtain coarser flour particle size (Pang *et al*., 2021).

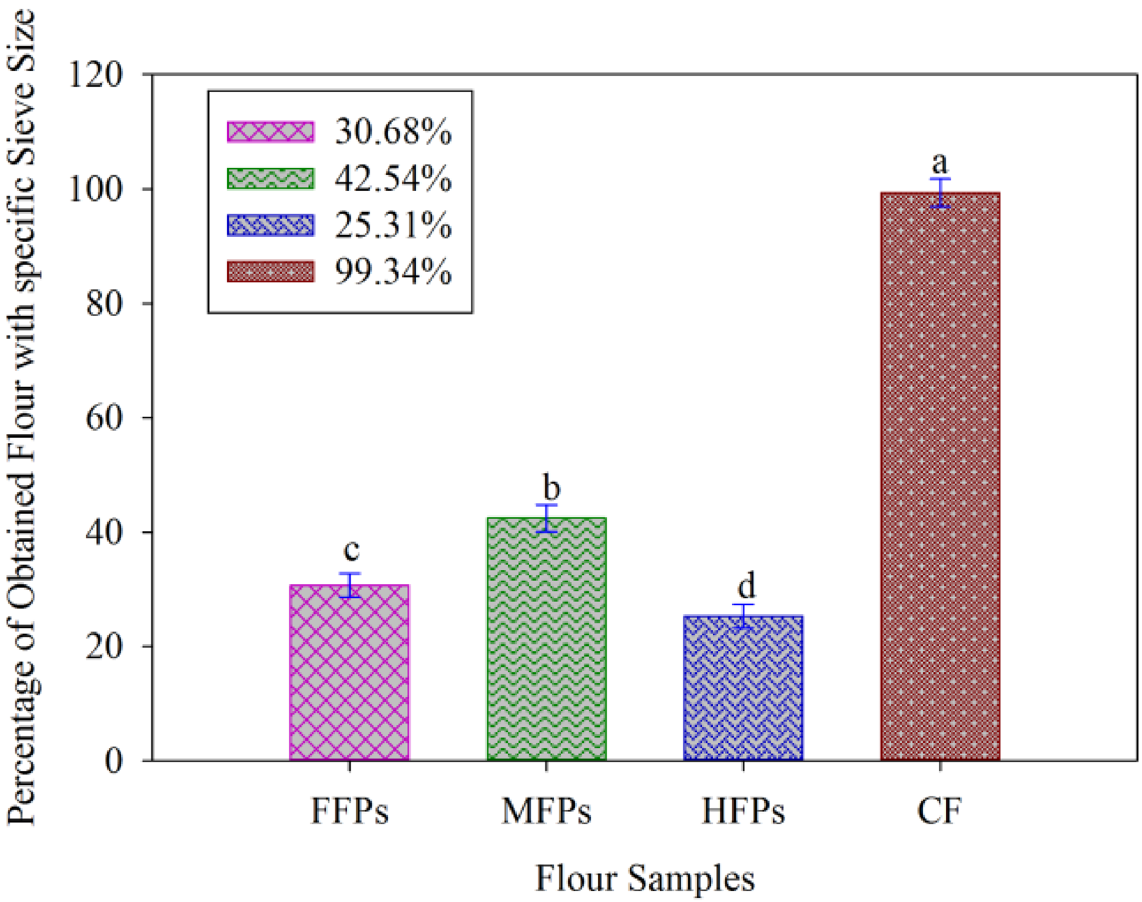


Figure 4.1. Graphical Representation of Flour Particle Size Distribution

4.1.2. Teff Flour Color as Influenced by Particle Size

Table 4.1 presents the color coordinates for the various teff flour particle sizes. The mean lightness medium and coarse particle size flour are not significantly different while fine flour particle size was significantly different ($p<0.05$) with the other particle sizes including the CF. Fine flour particle size (81.02) had significantly higher lightness followed by control flour (78.76) whereas medium and coarse flour particle size had (77.25) and (75.08) lightness respectively. The degree to which the bran of the teff grain broken and ground could likely is linked to such a particle size color effect. The bran part of the grain present in the coarse flour particle size decrease the lightness (L*) and increase the yellowness (a*) which indicates the presence of carotenoid pigments in the bran part of grain (Ahmed *et al.*, 2019). The L* value of flour particle size considerably increased as the particle size of teff flour decreased. This might be due to the smaller particles have finer pores and better homogeneity, which limit their capacity to absorb light and increase their capacity to reflect it, boosting the fine flour particle size whiteness. Comparable results were reported by Abebe, Collar, et al., (2015) and Assefa et al., (2018) who demonstrated the L* value of flour obtained from different teff variety and milling type in the range of 67.4 to 87.7. The redness or greenness (a*) and yellowness or blueness (b*) value of the flour of different particle size are 1.19, 12.03 and 1.42,10.87 for fine flour and control flour respectively while 1.51, 12.56 and 1.79,12.96 for medium and coarse flour particle size respectively. The results are slightly different from the work of Barretto et al., (2021) in case of color values of ivory teff flour milled using different milling methods.

The hue angle and chroma of the fine and control flour are not significantly different while the hue angle and chroma of the medium and coarse flour are significantly different ($p<0.05$). The findings were somewhat consistent with work by Abebe, Collar, et al., (2015) for two different teff variety mill types (h* 82.6, C* 15.4, and (h* 82.5, C* 15.0). Fine and control flour has more shine, while medium and coarse flour has less whiteness than fine and control. Differences in flour colour induced by particle size were barely apparent to the human eye.

Table 4.1. Color and water activity of Teff flour

Flours	L*	a*	b*	h*	C*	a_w at 24±0.20°C
FFPs	81.02±0.76[a]	1.19±0.08[c]	12.03±0.48[ab]	84.34±0.56[a]	12.09±0.47[ab]	0.45±0.01[a]
MFPs	77.25±1.08[bc]	1.51±0.11[b]	12.56±0.65[a]	82.56±0.28[b]	12.64±0.64[a]	0.44±0.01[b]
HFPs	75.08±0.97[c]	1.79±0.04[a]	12.96±0.38[a]	82.13±0.16[b]	13.08±0.38[a]	0.41±0.02[c]
CF	78.76±0.26[b]	1.42±0.08[b]	10.87±0.25[b]	83.14±0.73[ab]	10.96±0.26[b]	0.45±0.01[a]
P-value	0.000	0.000	0.003	0.003	0.002	0.008

FFPs, MFPs, HFPs, CF stands for fine, medium, higher or coarse flour particle size and control flour respectively. Data are expressed as the mean ± standard deviations; means that do not share a letter in the same column are significantly different ($P< 0.05$). L*, a*, and b* are CIE coordinates, h = hue, and C* = chroma

4.1.3. Flour Water Activity

The results stated in Table 4.1 showed that, water activity ($a_{w)}$ of fine flour (0.45) and control (0.45) are not significantly different. Medium flour particle size had lower water activity (0.44) than fine particle size and control flour whereas higher than coarse flour. The coarse flour particle size had the lowest water activity value (0.41) among all flour particle sizes. The difference is due to the protein and carbohydrate rich of fine and control flour over medium and coarse flour (Carter *et al*., 2015). Fine flour particle size was cohesive to have higher water activity over coarse flour. Hydration of macromolecules occurs when hydrophilic compounds, such as carbohydrates, interact and dissolve in water by creating hydrogen bonds with the water molecules (Landillon *et al*., 2008). When the particle size of teff flour is reduced, the surface area increases, allowing it to absorb more water. Fine flour are more protein rich than coarse flour, it was reported that water molecules may interact with hydrophilic regions of protein structure (Syamaladevi *et al*., 2016). Microbial growth can be difficult to control in foods and food materials with water activity greater than 0.6. The water activity of all treatment flour was less than 0.6, which is less susceptible for microbiological and biochemical deteriorations during storage.

4.1.4. Bulk Density of Flour

The bulk densities of different particle size of flour are significantly different as shown in table 4.2. Bulk density is clearly affected by particle size and moisture content (Saha *et al*., 2022). It is very important in determining the packaging requirement and material handling. Flour particle size of 500μm (0.77g/mL) had significantly ($p<0.05$) different from flour particle size of 180μm (0.68g/mL) and control (0.71g/mL). Medium (355μm) flour particle size had mean bulk density of 0.75g/mL which is significantly different from fine and control flour and not significant from coarse flour. The observations are closely agree with the reports of Y. Assefa et al., (2018) on disk mill (0.63g/ml), hammer mill (0.73g/ml) and blade mill (0.77 g/ml) flour. The difference comes from the loosely packed structure in fine flour particles to have lower bulk density and the denser accumulated granules found in the samples with coarser flour particle size with higher bulk density. More cohesive powders exhibit stronger gravitational attractive forces. This encourages particles to cluster in open areas and produces more gaps in the powder during pouring, which increases volume (H. W.

Woldemariam *et al*., 2021). Reduced particle size obtained lower bulk density. Tapping adds additional force to overcome these cohesive tendencies and causes particles to fall into void areas, reducing the volume of powder.

Table 4.2. Bulk Density and Functional properties of Teff flour Samples

Flours	Bulk density (g/mL)	WAC (ml/g)	WSI (%)	WAI (g/g)	OAC(ml/g)	SP (g/g)
FFPs	0.68±0.01[c]	1.25±0.04[a]	5.34±0.42[a]	5.41±0.31[a]	2.59±0.06[a]	9.48±0.44[a]
MFPs	0.75±0.01[a]	1.14±0.01[bc]	4.15±0.08[b]	5.11±0.62[c]	2.08±0.06[b]	6.58±0.20[b]
HFPs	0.77±0.01[a]	1.08±0.02[c]	3.90±0.56[c]	4.59±0.22[c]	1.77±0.09[c]	5.96±0.13[b]
CF	0.71±0.01[b]	1.16±0.01[b]	5.17±1.29[a]	5.24±0.08[b]	2.43±0.09[b]	8.95±0.26[a]
P-value	0.000	0.002	0.000	0.001	0.004	0.000

WAC= water absorption capacity, WSI= water solubility index, WAI= water absorption index, OAC=oil absorption capacity, SP=swelling power. Data are expressed as mean ± standard deviations; means that do not share letter on superscripts in the same column indicate statistically significant ($P < 0.05$). FFPs, MFPs, HFPs and CF are fine, medium, coarse flour particle size and control flour respectively

4.2. Functional Properties of Flour

Functional properties are basic characteristics that takes into account the intricate interactions between the composition, structure, molecular conformation, and physicochemical characteristics of food components as well as the environmental factors in which these are related to and measured. The structural quality, nutritional value, and acceptability of injera are all characterized by functional properties of flour (Dasa & Binh, 2019). The functional properties of different particle size of teff flour used in this study for injera formulation are showed in table 4.2. Water absorption is the amount of water needed to hydrate flour components to produce dough with optimum consistency (Sapirstein *et al*., 2018). The mean water absorption capacities of varied particle size of teff flour are 1.25ml/g, 1.14ml/g, 1.08ml/g and 1.16ml/g for fine, medium, coarse and control flour respectively. When the particle size of teff flour is reduced, the surface area increases, allowing it to absorb more water that exposed hydrophilic groups of fine powder interact with water. The protein content in the fine particle size is higher than other flour fractions.

The higher protein content of the fine flour might be increased hydrogen bonding thus facilitating water binding and entrapment. The oil absorption capacity of different particle size of teff flour was significantly different ($p<0.05$) as fine flour absorbs more oil than coarse flour. Accordingly the mean oil absorption capacities of fine, medium, coarse and control flour are 2.59ml/g, 2.08ml/g, 1.77ml/g, and 2.43ml/g respectively. Fine particle size and control flour had higher WAC and OAC than coarse and medium particle sizes due to the greater surface area and damaged starch to bind with water and oil molecules. On the other hand coarse flour particle size had lower oil absorption capacity. The difference might be due to the limited amount of non-polar protein side chains that would bind the side chains of hydrocarbons of the oil (Chandra *et al.*, 2015). The water absorption capacity of teff flour samples recorded in this work is greater than teff flour milled with different mill type (0.87 to 0.97ml/g) as indicated by Y. Assefa et al., (2018) and slightly agree with the results (0.89-1.06ml/g) reported by Abebe, Collar, et al., (2015) for variety and particle distribution of teff flour. The oil absorption capacity of the current result was in agreement with the observation of Y. Assefa et al., (2018) who reported the OAC from 2.30 to 2.5 ml/g. However, it was higher than the results (1.08-1.42ml/g) reported by Tsegaye, (2020).

Water absorption capacity (WAC) is crucial for uniformity and bulking of goods for use in baking(Abebe, Collar, *et al*., 2015). Flours having a high capacity to absorb oil may be advantageous in food items for preserving flavor, enhancing palatability, and extending shelf life of baked foods (Awuchi *et al*., 2019). High OAC makes the flour suitable in facilitating enhancement in mouthfeel when used in food preparations. Oil absorption capacity and Water absorption capacity are constraints that influence the consistency, texture, and mouthfeel of food products. Therefore fine flour particle size and control flour could have quality products than medium and coarse flour as they absorb more oil and water than that of medium and coarse flour.

The volume occupied by the gelatinized material is measured by the water absorption index (WAI) (Yousf *et al*., 2017) after swelling in much water, additional components such as carbohydrates and denatured protein preserving the consistency of the starch in the aqueous dispersion. The WAI and WSI of the fine flour was 5.41 and 5.34 respectively which are significantly different from medium and coarse flour samples which had 5.11, 4.15 and 4.59, 3.90 respectively. WAI and WSI of control flour were significantly different from both medium and coarse flour. The amount of polysaccharides that are released from the granule when too much water is added is determined by the water solubility index. Swelling power of fine flour particle size and control flour was statistically different from medium and coarse flour particle size. The differences are due to the fine granules amylolytically hydrolyzable and quickly hydrate. Increase in the value of WSI, WAI and SP results the dough is sticker and granules get better solubility during injera dough kneading for the good texture of injera (Y. Assefa *et al*., 2018). Therefore fine and control flour had high value of WSI, WAI and SP tended to get injera prepared from fine and control flour is soft, fluffy and easily rollable.

4.3. Pasting properties of Flour Samples

Pasting properties of starches of different particle size of teff flour samples are presented in table 4.3 and their pasting curves are displayed by figure 4.2. The pasting characteristics of flours were significantly ($p<0.05$) influenced by flour particle size. The mean values of peak viscosity of fine, medium, coarse flour particle size and control flour were 1056, 1030, 968 and 1067 respectively. The PV of fine particle size and control flour was higher as compared to medium and coarse flour particle size. The mean PV value of coarse flour is significantly different ($p < 0.05$) from fine and control. The variation of peak viscosity often associated with the swelling power of starch and the rate of disruption of the starch granules(Y. Assefa et al., 2018). Fine particle sizes of flour have more disrupted granules as compared to medium and coarse flour particle size (Abebe, Collar, *et al*., 2015). The results of the current finding was lower than the values reported by Y. Assefa et al., (2018) and Abebe, Collar, et al., (2015) from1544-1768mpas and 1304-1676mpas, respectively. The differences may be due to particle size of the flour. They also observed that teff flours with higher WSI and WAI have higher PV and BDV, which agreed with the current findings. The peak viscosity indicates the strength of pastes, which are formed during the gelatinization process in functional foods. It also reflects the extent of granule swelling and implies the viscous pile that is likely to be encountered during mixing (Liang & King, 2003). The swelling index increases with increasing peak viscosity, whereas low paste viscosity indicates increased solubility as a result of starch degradation or dextrinization (Mohammed *et al*., 2009). The fine and control flour particle size of the current study shows that the PV values are higher over medium and coarser that had increased swelling index.

The trough viscosity (TV) value of teff flour was significantly ($P< 0.05$) affected by flour particle size. The TV value of control flour was higher than the treatment samples. The flour particle sizes of fine, medium and coarse were not significantly different. The control flour had higher TV value than all flours with the value of 696.5 and the fine, medium and higher flour particle size had 549.5,572 and 636, respectively. The aggregate flour particle size had negatively correlated with the lower TV value that control flour is the aggregate of all flour particle size.

Coarse flour was significantly different from control flour which had lower BDV value of 271.5 which was lower than the other treatment flours. The Lower BDV value of coarse flour particle size implies the higher thermo-stability and lower shear thinning and disintegration of swollen systems (Abebe *et al*., 2015).

All treatment flour did not score significantly ($p>0.05$) different FV and SBV among them. This result indicates all flour particle size similarly recovering of viscosity in them during cooling period and the presence of closely similar amylose retrogradation. All flour particle size had related ability to form a viscous paste which is determined by the retro-gradation of soluble amylose in the process of cooling. SBV forecasts the degree of gels and the progressive retrogradation tendencies of the teff flour starch pasted system during cooling and storage. Pasting temperature indicates the minimum temperature required to cook starch. The PT of the teff flour was significantly influenced by particle size and it varied in the order of FFPs (76.27^{o}C) < CF (77.81^{o}C) < MFPs (81.90 ^{o}C) < HFPs (84.04 ^{o}C). Larger teff flour granules have a higher pasting temperature and lower swelling property. A higher pasting temperature also indicates that the flour has a higher structural rigidity (Aprianita *et al*., 2009). Coarse flour particle size has a higher structural rigidity followed by medium flour particle size. Y. Assefa et al., (2018) suggested flour with higher water solubility index tend to have lower pasting temperature ($p<0.05$) and this is in agreement with the current finding. The peak time of pasting properties was dependent on flour particle size that ranges from 5.11 min to 5.66min. The coarse flour particle size had higher peak time whereas fine flour particle size had lower peak time of pasting time.

Table 4.3. Pasting properties of teff flour as influenced by particle size.

Flour Sample	Pasting Property (RVA) Parameters						
	PV(cP)	TV(cP)	BDV(cP)	FV(cP)	SBV(cP)	PT(^oC)	Pt(min)
FFPs	1056±38[a]	549.5±74[b]	436±42[ab]	1278.5±39[a]	852.5±31[a]	76.27±0.38[c]	5.11±0.18[c]
MFPs	1030±17[ab]	572±39[ab]	457.5±23[ab]	1345.5±202[a]	842.5±39[a]	81.90±2.80[ab]	5.43±0.10[ab]
HFPs	968±20[b]	636±10[ab]	271.5±170[b]	1344±7[a]	711.5±118[a]	84.04±2.41[a]	5.66±0.07[a]
CF	1067±40[a]	696.5±73[a]	517.5±34[a]	1493±47[a]	796±128[a]	77.81±1.23[bc]	5.36±0.04[bc]
P-value	0.017	0.044	0.048	0.163	0.082	0.004	0.002

Values are mean ± standard deviation. Means that do not share a letter within the same column are significantly different at $P<0.05$. Where: - PV= Peak viscosity, TV= Trough viscosity, BDV= Breakdown viscosity, FV= Final viscosity, SBV= Setback viscosity, PT= Pasting temperature and Pt= Pasting time (peak time of pasting). FFPs, MFPs, HFPs, CF stands for fine, medium, higher or coarse flour particle size and control flour, respectively.

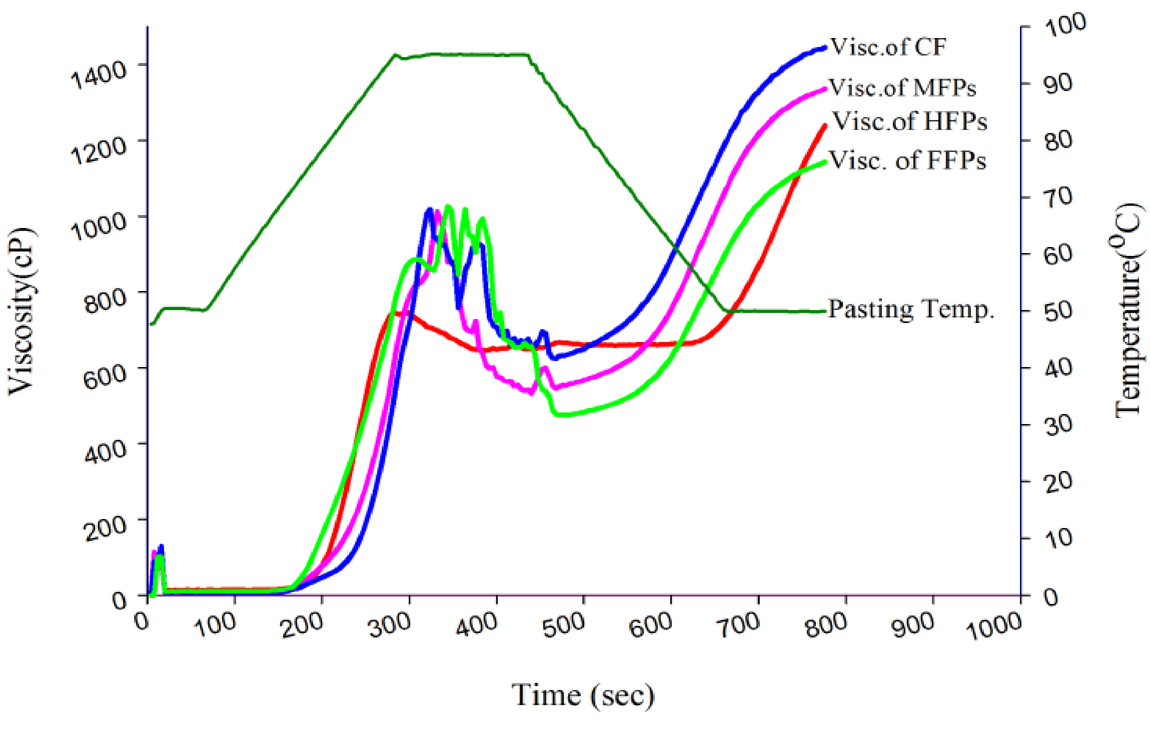


Figure 4.2. Pasting Curves of Flour Samples

4.4. Rheological Properties

The visco-elastic properties of varied teff flour particle size dough are presented in the table 4.4. The values of elastic (storage) modulus G' and viscous (Loss) modulus G" at a frequency of 1Hz was significantly different ($P< 0.05$). The elastic (3464.02) and viscous moduli (1654.83) were higher in coarse flour particle size followed by medium flour particle size (1656.59) and 499.38 respectively. Higher elastic moduli, G', dough may not be suited for injera production because the carbon dioxide in the batter must easily escape, aiding the creation of the requisite evenly spaced injera eyes (Y. Assefa *et al*., 2018). Despite the wide range of values, storage and loss modulus follow a similar curve to the power curve after the elastic network is built up, probably due to protein interactions (Niveditha, 2019). The viscoelastic moduli of dough formed with coarse flour particle size were much larger than those observed with medium flour particle size, fine flour particle size, and control flour.

The viscous modulus (G") of the dough obtained from all flour samples were closer to the elastic modulus (G'), as measured by the loss tangent (G"/G') that was not significantly different. The Tan δ (G"/G') value of control flour dough was 0.54 and the values of coarse, medium, and fine flour particle sizes 0.49, 0.32, and 0.28, respectively. The values indicating that the dough had elastic-like behaviour. It was used to estimate the loss factor of batter which had elastic behavior. The better shape retention provided by the major elastic function during handling and cooking suggests that the matrix structure is not weak and easily deformable (H. W. Woldemariam *et al*., 2021). All flour samples had G'> G" resulting in (tan δ) <1, showing that the gels produced from all flour samples behaved firmly (Abebe & Ronda, 2014).

Table 4.4. Effect of flour particle size on viscoelastic properties of dough at Angular Frequency of 36 rad/s.

Flour Particle Size	G' (elastic or storage module) (Pa)	G" (viscous or loss modulus)(Pa)	Tan δ (G"/G')	Shear Stress(Pa)	Shear Strain(Pa)
FFPs	1540.86±143.3[c]	423.7±172.9[c]	0.28±0.14[a]	262.67±2.94[a]	331.02±23.03[b]
MFPs	1656.59±277[b]	499.38±243[b]	0.32±0.20[a]	270.34±1.91[a]	877.95±466.77[a]
HFPs	3464.02±1861[a]	1654.83±814[a]	0.49±0.03[a]	258.31±9.87[a]	443.77±111.27[b]
CF	400.23±383[d]	246.84±240[d]	0.54±0.13[a]	222.68±44.09[a]	997.94±244.83[a]
P-value	0.028	0.018	0.148	0.123	0.043

Means that do not share a letter on superscript in the same column are significantly different. Where FFPs= fine flour particle size (0-180µm), MFPs= medium flour particle size (181-355µm), HFPs=higher flour particle size (356-500µm) and CF= control flour

4.5. Proximate Composition of Teff Flour

Three teff fractions (500µm, 355µm and 180µm) and control flour used as raw material for injera preparations were assayed for proximate composition. The results are presented in Table 4.5. The proximate composition of flour was significantly ($p < 0.05$) affected by flour particle size.

4.5.1. Flour Moisture Content

The moisture content of fine flour particle size and control flour is 9.04±0.0.07% and 9.05±0.0.04% respectively was not significantly different. Medium particle size 8.98±0.06% and coarse particle size 8.79±0.06% has lower moisture content than that of fine and control flour. The results are close agreement with the values reported by Abebe, Collar, et al., (2015)10.3 ± 0.1 and Y. Assefa et al., (2018) 10.9±1.2% for the fine and control flour. Compared to fine particle size and control flour; medium and coarse particle size flour have significantly lower moisture contents. The reason of the difference is that the germ and endosperm of grain hold more moisture than that of bran. This implies fine flour particle size is mostly obtained from the germ and endosperm while higher particle size is highly contains brans that do not have more moisture either dried or cannot hold more moisture initially.

4.5.2. Crude Protein Content of Flour

The protein content of three flour particle size and control flour was significantly ($p<0.05$) different (table 4.4). Control flour had higher protein content (11.27%) than that of fine (10.07%), medium (9.90%) and coarse (9.12%). These results were in line with the findings of Bultosa (2007) and Assefa et al., (2018), who reported the protein content from 9.30 to 11.07% and 11%, respectively. A proteins fraction in teff flour was mostly distributed in the endosperm part of the grain that was obtained more in the fine flour over coarser one. The differences in protein composition among teff flours of different particle size may be ascribed to the portion of grain to be milled to different flour particle size.

4.5.3. Crude Fat Content of Flour

The fat content of teff flour of different particle size ranged from 2.54% to 3.00%. The fat content of fine particle size (3.00%) is the highest value than control (2.70%), medium (2.58%) and coarse flour particle size (2.54%). This result was in line with the observation of Baye (2014), who reported the flour fat content of 2.5%. The differences in fat content among teff flours of different particle size may be ascribed to the portion of grain to be milled to different flour particle size as protein. The fine flour particle size had a larger fat content than coarse flour. This might be due to the high degree of grain germ introduction in the fine flour. Increasing fat content for the quinoa flour was reported earlier when the particle size was reduced (Ahmed *et al*., 2019).

4.5.4. Crude Fiber Content of Flour

The crude fiber content of flour particle size ranges from 2.42% to 3.81% d.m. The coarse flour particle size was found to be with the highest fiber content 3.81% followed by medium, control flour and fine particle size (3.71%), 3.52% and 2.42%), respectively. More bran fractions in Coarse flour particle size resulted higher fiber content (Bassi *et al*., 2021).

4.5.5. Crude Ash Content of Flour

The ash content was significantly ($p<0.05$) affected by the flour particle size. The total ash content of FFPs, MFPS, HFPs and CF are 2.02%, 2.45%, 2.61% and 2.44% respectively. The ash content of food products is a measure of the total amount of minerals in the foods produced (Ibeabuchi *et al*., 2017). The amount of ash content is relatively higher in coarse flour particle size (2.61%) as compared to the medium (2.45%), control (2.44%) and fine flour particle size (2.02%). The total ash content value of these flour particle sizes had been found in accordance with the reported values (2.54-2.63%) as determined previously for teff (Abebe, 2015; Bultosa, 2007). The differences in ash content among different particle size of flour may be due to the incorporation of more bran into the coarse and medium flour particle size (Ibeabuchi *et al*., 2017).

4.5.6. Carbohydrate Content of Flour

One of the most vital ingredients in many diets is carbohydrate. It can exist as alone molecules, in physical association with other molecules, or chemically bonded to other molecules (BeMiller, 2010). The flour particle size had a significant ($p<0.05$) effect on the carbohydrate content of flour and the values varied from 71% to 74%. The carbohydrate content was highest in coarse flour particle size (74.11%) followed by fine (73.30%), and medium flour particle size (72.82%), lowest in control flour (71.69%). A similar result was reported by Agza et al., (2018), who observed the carbohydrate content 71.43%.

Table 4.5. Proximate composition of teff flour as influenced by particle size.

Flour Sample	Moisture (% db)	Crude protein (db%)	Crude fat (db %)	Total ash (db %)	Crude fiber (db %)	Carbohydrate (db%)
FFPs	9.04 ± 0.07^{a}	10.07 ± 0.05^{b}	3.00 ± 0.04^{a}	2.02 ± 0.01^{c}	2.42 ± 0.01^{c}	73.30 ± 0.06^{b}
MFPs	8.98 ± 0.06^{a}	9.90 ± 0.07^{c}	2.58 ± 0.06^{b}	2.45 ± 0.02^{b}	3.71 ± 0.02^{a}	72.82 ± 0.26^{c}
HFPs	8.79 ± 0.06^{b}	9.12 ± 0.01^{d}	2.54 ± 0.15^{b}	2.61 ± 0.01^{a}	3.81 ± 0.07^{a}	74.11 ± 0.15^{a}
CF	9.05 ± 0.04^{a}	11.27 ± 0.07^{a}	2.70 ± 0.09^{b}	2.44 ± 0.01^{b}	3.52 ± 0.06^{b}	71.69 ± 0.09^{d}
P-value	0.002	0.000	0.001	0.000	0.000	0.000

Means that do not share a letter on superscript in the same column are significantly different. Where FFPs= fine flour particle size (0-180μm), MFPs= medium flour particle size (181-355μm), HFPs=coarse flour particle size (356-500μm) and CF = control flour.

4.6. Mineral Contents of Flour Samples (Ca, Fe and Zn)

The iron, calcium, and zinc content of the flour samples of fine, medium, coarse and control is shown in figure 4.3. The effect of flour particle size on the mineral contents of flours were investigated as there was no significant difference in iron and zinc content of control flour, medium and coarse flour particle size (13.95 mg/100g and 1.65 mg/100g, 14.80mg/100g and 1.82 mg/100g, 14.62 mg/100g and 1.93 mg/100g), respectively. Fine flour particle size had lower iron (11.57 mg/100g) and zinc (1.32 mg/100g) content as compared to other particle sizes. The highest average calcium content (144.47mg/100g) was recorded for coarse flour particle size followed by control flour (141.98 mg/100g). Fine and medium flour particle was recorded with lower values of (125.83 mg/100g and 133.33 mg/100g) respectively. According to Atwell & Finnie, (2016), the higher bran content in coarse flour increase the mineral contents while the higher content of endosperm and germ content of fine flour lower the mineral contents. Calcium, which is present in teff in very high concentrations, was reported 142(g/100 g), iron 11(g/100 g), Zinc 2.2(g/100 g) for Tseday variety by Shumoy et al., (2017).

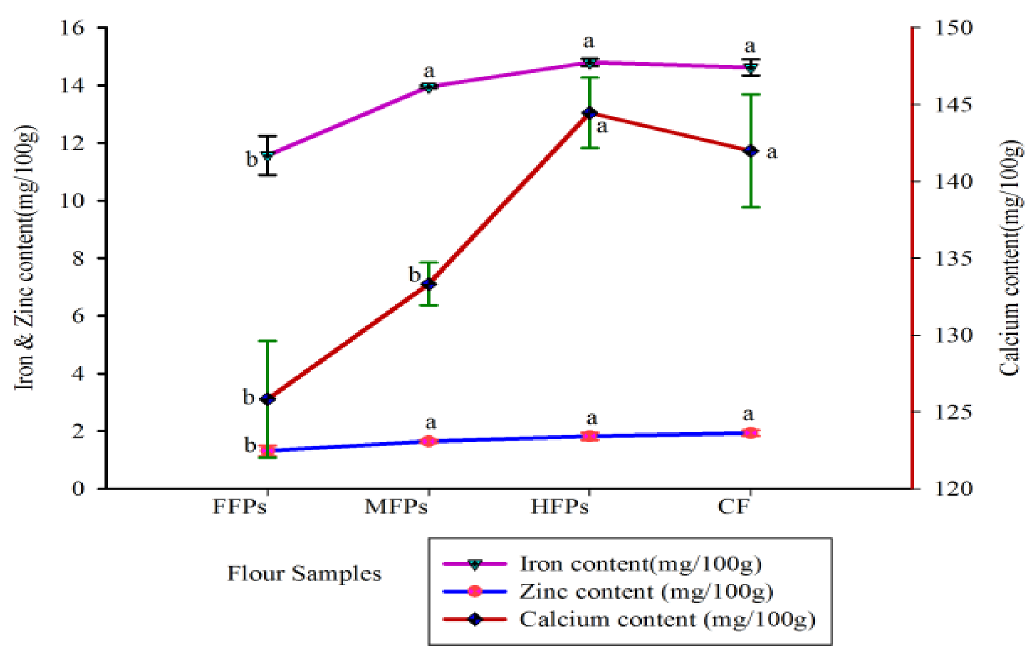


Figure 4.3. Mineral contents of flour as influenced by particle size.

4.7. Total Phenolic Compounds and Anti-nutritional Contents of Flour Samples

4.7.1. Total Phenolic Compounds of Flour

The total phenol content of fine, medium, higher/coarse flour particle size and control flours were 88.00mg GAE/100g, 91.04mg GAE/100g, 98.63 mg GAE/100g and 96.67 mg GAE/100g, respectively as presented in table 4.6. Total phenol and anti-nutritional factors are compounds found in greater concentrations in the grain's outer layers (Yisak *et al*., 2022). The total phenolic content was statistically significant ($p<0.05$) within the flour particle size. The highest and the lowest TPC were observed in coarse and fine flour particle sizes, respectively. The difference might be due to the level of inclusion of outer layer part of the grain into the flour. Coarse flour particle size largely came from the outer layer of the grain whereas the fine flour particle size came from the interior part of the grain.

4.7.2. Condensed Tannin of Flour

The condensed tannin content of fine, medium, higher/coarse flour particle size and control flours were 9.30 mg catechin equiv. /100g, 10.58 mg catechin equiv./100g, 12.98 mg catechin equiv./100g and 11.19 mg catechin equiv./100g, respectively. The higher condensed tannin content was observed in coarse flour particle size and the lower condensed content was observed in fine flour particle size. This might be due to the high concentration of condensed tannin in the testa cell walls and pericarp region of the teff grain, which resulted in a distinct proportion of fine and coarse flour (Xiong *et al*., 2019).

4.7.3. Phytic Acid Content of Flour

Flour particle size had significant ($P<0.05$) effect on phytate content of flours as shown on table 4.6. The coarse flour particle size had the highest phytate content 447.41mg/100g followed by control (412.08 mg/100g), medium (391.01 mg/100g) and (249.03 mg/100g). Reducing flour particle size from 500μm to 180μm reduces the phytate content with 39.68%. In teff grain, phytic acid is largely distributed in external layers of pericarp and aleurone layer. Larger particle sizes of bran are obtained from outer bran layers and hence contain higher phytic acid content compared to the smaller particle sizes (Majzoobi *et al*., 2014). The result was in agreement with the teff flour phytate contents (437.65 mg/100g) reported by Anberbir et al., (2023).

Table 4.6. Total phenolic compounds and anti-nutritional contents of flours.

Flour Samples	Total Phenol (mg GAE/100g)	Condensed Tannin (Catechin Equiv.mg/100g)	Phytates (mg/100g)
FFPs	88.00±4.15^{d}	9.30±1.04^{c}	249.03±12.91^{d}
MFPs	91.04±3.06^{c}	10.58±1.42^{bc}	391.01±6.29^{c}
HFPs	98.63±5.47^{a}	12.98±3.36^{a}	447.41±5.87^{a}
CF	96.67±2.65^{b}	11.19±2.55^{b}	412.08±3.07^{b}
P-value	0.000	0.001	0.000

Means that do not share a letter on superscript in the same column are significantly ($p<0.05$) different. Where FFPs= fine flour particle size, MFPs= medium flour particle size, HFPs=higher flour particle size and CF= control flour

4.8. Effect of Flour Particle Size and Dough Kneading Time on pH, Titratable Acidity and Viscosity of Batter

The pH and titratable acidity of a food product indicate its sourness. The sour taste of injera is a unique sensory characteristic. The interaction of flour particle size and dough kneading time had significant ($p<0.05$) effect on the pH, TA and batter viscosity as shown in the figure 4.4. The pH value of injera batter was ranged from 3.75 to 4.33. The highest pH value (4.33) was recorded from coarse flour particle size with 12 minute dough kneading time, while the lowest pH value was observed from control flour batter with 4 minute dough kneading time (3.75). Earlier research on different brands of injera revealed that the pH of the injera samples ranged between 3.50 and 4.5 (Attuquayefio, 2015). Batter from fine flour particle size and control flour had lower pH value than medium and coarse flour particle size. The difference might be due to the difference of fermentable starch content in the flour. Lactic acid production during fermentation was dependent on sugar amount in the raw material (Attuquayefio, 2015).

Decrease with lower rate in pH was observed due to change in kneading time. As the kneading time increase from 4 minute to 12 minute, the pH of control flour batter decrease from 3.98 to 3.75. The pH of fine flour particle size batter decreased from 3.95 to 3.87 with kneading time increased from 4 minute to 12 minute. The pH value of medium and coarse flour particle size batter also decreased from 4.10 to 3.93 and 4.33 to 4.02 respectively as kneading time increased from 4 minute to 12 minute. The differences could be due to a difference in kneading time, which could impact fermentation kinetics (Assefa *et al*,.2018). Kneading increased the fermentation and maximizes the acidity of batter *(Tsatsaragkou et al*., 2023).

The TA of batter samples scored from 1.27 to 1.63 as the result of flour particle size and dough kneading time interaction effect (figure 4.4a). The highest TA value (1.63) was observed from the fine and medium flour particle size batter sample kneaded at 12 minute while the lowest TA value (1.27) was obtained from the coarse flour particle size batter sample kneaded at 4 minute. The titratable acidity of the batter increased significantly ($p<0.05$) as the kneading time increased. This increase in TA could be attributed to the rapid decrease in pH as kneading time increased (Desiye *et al*., 2017).

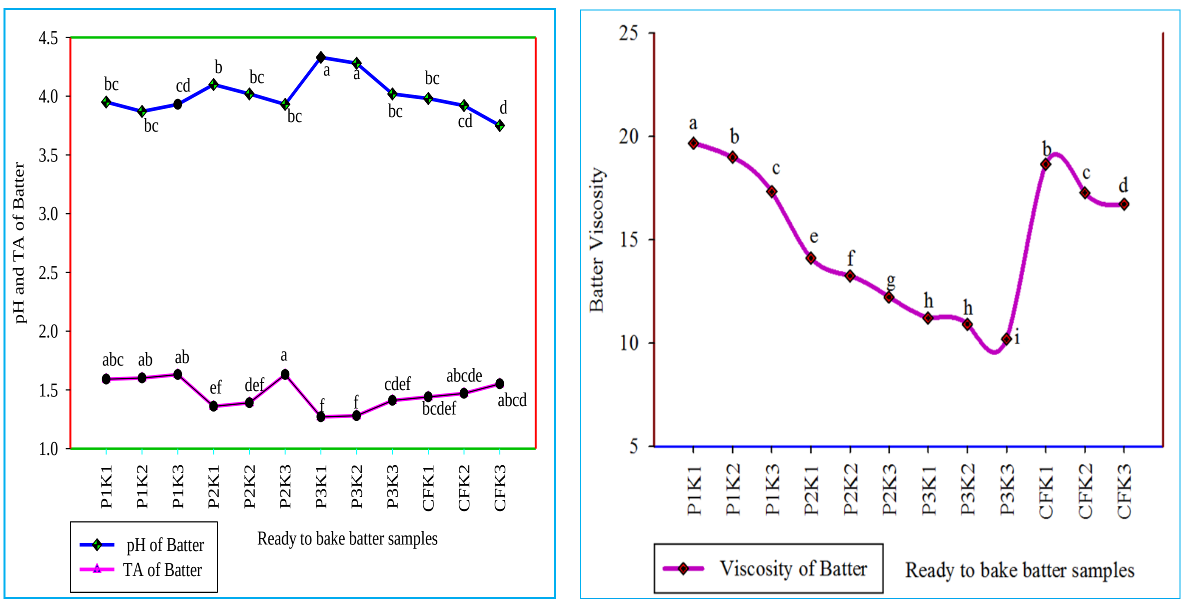


Figure 4.4. Batter viscosity and change in pH and TA due to flour particle size and dough kneading time.

Where: P1, P2 and P3 are fine, medium and coarse flour particle size and CF is control flour with K1, K2 and K3 are dough kneading time 4, 8 and 12 minutes.

A measurement of batter viscosity of the samples was made at each baking time. Figure 4.4 shows what appears to be a viscosity of batter at 60 hours first phase fermentation and 6 hours second phase of fermentation with varied kneading time of dough. The batter viscosity was significantly ($p<0.05$) different as a result of flour particle size and dough kneading time interaction effect. The batter sample obtained from fine flour particle size at 4 minute dough kneading time had highest (19.68cP) viscosity while the batter sample obtained from coarse flour particle size with 12 minute dough kneading time had lowest (10.18cP) viscosity. Both flour particle size and dough kneading time had significant effect on batter viscosity. Viscosity of batter increased with decreased flour particle size and decreased with dough kneading time. The difference was due to the higher gas evolution (increase in air bubbles) observed during long kneading time. Fine flour absorbs more water than coarse flour that helps to make thick batter in lower particle size of flour than coarser one. As a result the viscosity of finer flour was recorded higher than the viscosity of coarser (Sun *et al*., 2022).

4.9. Instrumental Texture Profile of Injera as Affected by Flour Particle Size and Dough Kneading Time

Acceptable injera is soft, fluffy, and rollable without cracking which should able to keep these textural qualities up to three days of storage (Yasin, 2021). The mean of compression force required for the teff injera cutting through the two primary parameters; flour particle size and dough kneading time, as shown in Table 4.7, The means of compression force required for cutting injera due to flour particle size was 17.48 and 17.20 for coarse and medium particle size respectively on the 1st day storage. The compression force required to cut injera due to dough kneading time was decreased with kneading time thus 17.21, 15.97, 13.82 for 4, 8 and 12 minute kneading time, respectively. On the second day the compression force required to cut injera was in the range of 12.02 to 16.17. There is no significant different due to kneading time in the 2nd day storage while on the 3rd day storage the compression force required to cut injera was declined from 15.25 to 11.42 from coarse flour particle size to control flour injera. There is also significant ($p<0.05$) difference for dough kneading time as the values scored was 14.96,12.76 and 12.40 for 4, 8 and 12 minute kneading time, respectively. Texture profile analysis deals with the evaluation of mechanical properties by subjecting a material to controlled force and generating the deformation curve of its response.

Table 4.7. Instrumental measurement of injera texture due to variation of the main effect.

Flour Particle size	Texture of 1st Day Storage in F/N	Texture of 2nd Day Storage in F/N	Texture of 3rd Day Storage in F/N
FFPs	14.82±1.34^{b}	12.71±0.71^{b}	11.61±1.03^{b}
MFPs	17.20±1.29^{a}	15.66±1.82^{a}	15.20±1.27^{a}
HFPs	17.48±1.08^{a}	16.17±1.28^{a}	15.25±1.79^{a}
CF	13.17±0.69^{b}	12.02±0.64^{b}	11.42±0.96^{b}
P-Value	0.000	0.000	0.000
Dough Kneading time			
4 min	17.21±1.15^{a}	15.00±3.05^{a}	14.96±1.00^{a}
8 min	15.97±1.66^{a}	13.74±1.35^{a}	12.76±1.34^{b}
12 min	13.82±0.42^{b}	13.68±0.97^{a}	12.40±0.53^{b}
P-value	0.000	0.051	0.001

Means that do not share a letter on superscript in the same column are significantly different. Where, FFPs= fine flour particle size (180µm), MFPs=medium flour particle size (355µm), HFPs=higher flour particle size (500µm) and CF= control flour

The interaction of flour particle size and dough kneading time had a significant ($p<0.05$) effect on the firmness and compression force to cut injera samples. The highest cutting force value (19.92N, 18.51N, and 16.44N) was obtained for injera made from coarse flour particle size kneaded at 4 minute while the lowest value (11.33N, 11.12N, and 10.04N) was recorded for injera prepared from control flour kneaded at 12 minute kneading time on the 1st, 2nd and 3rd days of storage. The prepared injera from coarse flour particle size with all dough kneading time was more firm due to the larger flour particle size.

The instrumental texture analysis indicates that injera prepared from fine particle size and control flour requires lower compression force to cut. This conclude that soft injera require less force to cut. The higher firmness of food was considered to be caused by starch retrogradation and moisture dispersion (C. Lee *et al.*, 2008). The compression force to cut injera made from all types of flour particle size with all kneading time decreases with storage day increase as indicated on the figure 4.5. This might be due to the staling of injera which cause to lose its elasticity (Yetneberk *et al*., 2004). Since stale injera is significantly harder and firmer than fresh injera, it is easily broken and requires less force to cut than fresh injera (Yaregal *et al*., 2022).

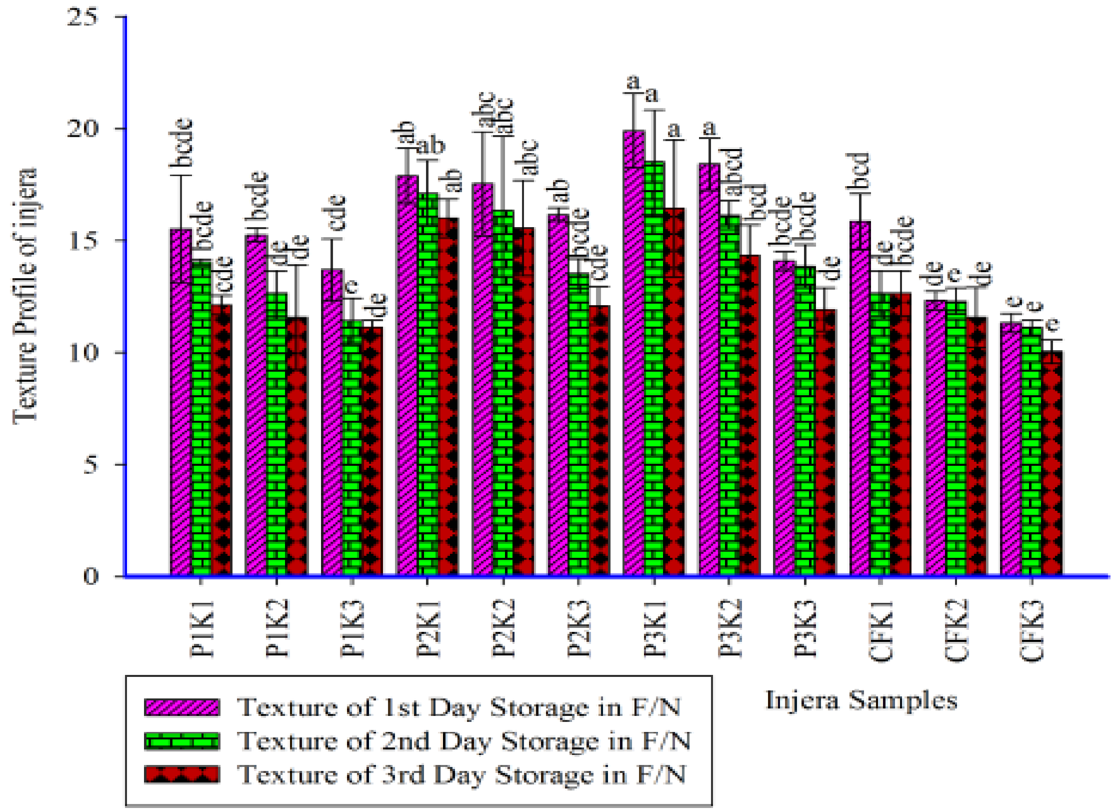


Figure 4.5. Texture profile of injera samples on the day 1, 2 and 3 storage.

4.10. Effect of Flour Particle Size and Dough Kneading Time on Color of Injera

Color is a significant factor in characterizing baked goods. Color is expressed as three values by the CIE-L*ab (Commission Internationale d''Eclairage), L* for lightness, a* for redness, and b* for yellowness (Carbas *et al*., 2016). A lower L* value indicates a darker, a positive a* value indicates redness, and a positive b* value indicates yellowness. Color qualities of injera reveal the first impression prior to eating whether to like or dislike the product. When injera is processed, a non-enzymatic reaction determines the colour combination based on the protein and carbohydrate composition of the flour. Due to caramelization during baking, flour with higher protein and carbohydrate content exhibited a darker colour (low L*). The product's eye distribution can be determined by the difference in colour between the injera's surface and hole (Cherie *et al*., 2018).

The color characteristics (L*, a* and b*) of injera samples at time of baking and 1st day storage were presented in Table 4.8 and 4.9 and showed a significant ($p<0.05$) effect on both front and back surfaces. The color of the front and back of the injera samples on the storage day and for the first day after storage was significantly affected ($p< 0.05$) by the interaction of the flour particle size and dough kneading time. The lowest L* value was recorded on injera samples formulated from control flour with 12 minute kneading time for front surface at time of baking. The highest L* value was obtained on injera samples made from fine flour particle size kneaded for 12 minute and the front surface of injera at the time of baking. However the L*, a* and b* values of injera samples from front surface on the day of baking was varied from 42.20 to 49.54, 2.65 to 4.06 and 14.45 to 17.87, respectively. The hue angle and chroma of the injera samples on the day of baking was ranged from 77.20 to 79.56 and 14.70 to 18.33 respectively.

The L* value of injera samples was increased on the back surface as a*, b*, h* and c* values are varied differently. The L* values ranged from 52.20 to 55.61 at the time of baking and the a*, b*, h* and c* values are in the range of 2.52 to 3.76, 14.66 to17.30, 78.37 to 80.47 and 15.00 to 17.64, respectively. Changes in colour reveal the extent of browning processes during the injera baking process such as caramelization, the Maillard reaction, the level of heating, and pigment degradation (Pathare *et al*., 2013).

The highest mean of (L*) values from front surface was observed on injera samples made from coarse flour particle size kneaded for 12 minute (48.81). The lowest (L*) values from front surface was observed on injera samples prepared from medium flour particle size kneaded for 8 minute (39.74). The values of a*, b*, h* and c* are in the ranges of 1.36 to 2.84, 7.97 to 13.16, 74.33 to 80.31 and 8.09 to 13.46, respectively. The L*, h* and b* values of injera were higher at the back surface while a* values are lower than the values of front surface as of day one storage.

Table 4.8. Interaction effect of main factors on the color of the injera on the day of baking.

Factors: FPs	Factors: DKt	Face	L*	a*	b*	h*	c*
FFPs	4min	Front	48.02±0.05^{c}	3.28±0.04^{de}	15.82±0.08^{ef}	78.28±0.10^{bc}	16.16±0.08^{de}
		Back	53.90±0.14^{c}	3.36±0.06^{a}	16.34±0.09^{c}	78.37±0.15^{b}	16.68±0.09^{c}
	8min	Front	45.97±0.13^{e}	2.66±0.04^{f}	14.45±0.06^{h}	79.56±0.13^{a}	14.70±0.07^{g}
		Back	54.82±0.04^{b}	3.13±0.05^{ab}	16.73±0.22^{b}	79.41±0.05^{ab}	17.02±0.23^{b}
	12min	Front	49.54±0.55^{b}	3.66±0.25^{b}	17.16±0.19^{bc}	77.96±0.68^{bcd}	17.55±0.24^{b}
		Back	53.86±0.06^{c}	2.52±0.03^{c}	14.78±0.07^{f}	80.34±0.07^{a}	15.00±0.07^{f}
MFPs	4min	Front	46.82±0.03^{d}	3.57±0.11^{bc}	16.92±0.07^{cd}	78.07±0.31^{bcd}	17.29±0.09^{bc}
		Back	53.86±0.06^{c}	2.52±0.03^{c}	14.78±0.07^{f}	80.34±0.07^{a}	15.00±0.07^{f}
	8min	Front	46.06±0.10^{de}	3.19±0.05^{e}	15.62±0.04^{fg}	78.46±0.18^{b}	15.94±0.04^{ef}
		Back	52.20±0.19^{d}	2.77±0.10^{bc}	14.91±0.06^{f}	79.48±0.41^{ab}	15.16±0.04^{f}
	12min	Front	51.37±0.66^{a}	3.52±0.06^{bcd}	17.20±0.05^{b}	78.44±0.20^{b}	17.55±0.05^{b}
		Back	54.09±0.07^{c}	2.63±0.04^{bc}	15.54±0.08^{e}	80.41±0.11^{a}	15.76±0.08^{e}
HFPs	4min	Front	48.48±0.03^{c}	4.06±0.02^{a}	17.87±0.03^{a}	77.20±0.06^{d}	18.33±0.03^{a}
		Back	55.45±0.14^{a}	2.68±0.03^{bc}	15.95±0.12^{d}	80.45±0.03^{a}	16.18±0.13^{d}
	8min	Front	48.44±0.06^{c}	3.64±0.06^{b}	17.10±0.07^{bc}	77.98±0.24^{bcd}	17.49±0.05^{bc}
		Back	52.24±0.04^{d}	2.62±0.04^{c}	15.44±0.06^{e}	80.47±0.03^{a}	15.66±0.06^{e}
	12min	Front	47.74±0.06^{c}	3.76±0.07^{b}	16.82±0.12^{d}	77.39±0.30^{cd}	17.23±0.11^{c}
		Back	55.61±0.03^{a}	3.01±0.57^{abc}	14.66±0.05^{f}	78.43.15^{b}	14.98±0.13^{f}
CF	4min	Front	48.07±0.09^{c}	3.33±0.08^{cde}	15.90±0.07^{e}	78.18±0.33^{bc}	16.24±0.05^{d}
		Back	53.91±0.03^{c}	3.36±0.05^{a}	16.34±0.07^{c}	78.37±0.19^{b}	16.68±0.06^{c}
	8min	Front	45.94±0.18^{e}	2.65±0.09^{f}	14.50±0.14^{h}	79.63±0.42^{a}	14.74±0.12^{g}
		Back	54.94±0.06^{b}	3.37±0.05^{a}	16.74±0.06^{b}	78.63±0.19^{ab}	17.08±0.06^{b}
	12min	Front	42.20±0.18^{f}	3.24±0.05^{de}	15.35±0.08^{g}	78.09±0.25^{bcd}	15.69±0.07^{f}
		Back	55.00±0.12^{a}	3.41±0.03^{a}	17.30±0.05^{a}	78.85±0.12^{ab}	17.64±0.05^{a}
P-value of front			0.000	0.000	0.000	0.003	0.000
P-value of back			0.000	0.000	0.000	0.001	0.000

Means that do not share a letter on superscript in the same column are significantly different. Where, FFPs= fine flour particle size (180µm), MFPs= medium flour particle size (355µm), HFPs=higher flour particle size (500µm) and CF= control flour

Table 4.9. Interaction effect of main factors on the color of the injera of 1st day storage.

Factors		Face	L*	a*	b*	h*	c*
FPs	DKt						
FFPs	4min	Front	39.76±0.17^{de}	2.01±0.22^{bc}	9.71±0.55^{de}	78.18±0.55^{ab}	9.91±0.58^{de}
		Back	56.61±0.18^{abc}	1.35±0.05^{bc}	10.44±0.11^{cd}	82.65±0.18^{ab}	10.52±0.11^{cd}
	8min	Front	44.57±0.93^{b}	1.65±0.10^{cd}	8.92±0.14^{efg}	79.52±0.47^{ab}	9.07±0.16^{efgh}
		Back	57.88±0.39^{ab}	1.27±0.04^{c}	10.39±0.01^{cd}	83.05±0.19^{ab}	10.47±0.02^{cd}
	12min	Front	40.21±1.68^{cde}	1.36±0.05^{d}	7.97±0.54^{g}	80.31±0.30^{a}	8.09±0.55^{h}
		Back	56.37±0.70^{abc}	1.22±0.08^{c}	10.45±0.25^{cd}	83.31±0.58^{a}	10.52±0.24^{cd}
MFPs	4min	Front	44.37±0.13^{b}	2.42±0.01^{ab}	11.47±0.01^{bc}	78.09±0.05^{ab}	11.72±0.01^{bc}
		Back	52.43±0.22^{ef}	1.27±0.03^{c}	9.60±0.07^{ef}	82.48±0.23^{ab}	9.69±0.07^{ef}
	8min	Front	39.74±0.43^{de}	2.01±0.02^{bc}	9.55±0.09^{e}	78.09±0.13^{ab}	9.76±0.09^{ef}
		Back	51.86±0.73^{f}	1.13±0.02^{c}	9.04±0.02^{f}	82.85±0.14^{ab}	9.11±0.02^{f}
	12min	Front	42.86±0.25^{bc}	2.40±0.01^{ab}	11.51±0.25^{bc}	78.22±0.24^{ab}	11.76±0.24^{bc}
		Back	50.59±1.10^{f}	1.50±0.06^{bc}	10.16±0.10^{de}	81.60±0.23^{abc}	10.27±0.11^{de}
HFPs	4min	Front	48.75±0.18^{a}	2.84±0.06^{a}	13.16±0.04^{a}	77.81±0.30^{ab}	13.46±0.03^{a}
		Back	55.12±0.15^{bcde}	2.17±0.02^{a}	13.12±0.13^{a}	80.61±0.01^{abc}	13.30±0.13^{a}
	8min	Front	43.33±0.04^{b}	2.38±0.13^{ab}	10.78±0.16^{cd}	77.52±0.84^{ab}	11.04±0.13^{cd}
		Back	58.45±0.70^{a}	1.46±0.04^{bc}	10.79±0.33^{bc}	82.29±0.35^{ab}	10.89±0.33^{bc}
	12min	Front	48.81±0.43^{a}	2.58±0.09^{ab}	12.38±0.08^{ab}	78.24±0.31^{ab}	12.65±0.09^{ab}
		Back	55.77±0.15^{abcd}	1.55±0.03^{bc}	11.06±0.16^{b}	81.74±0.47^{abc}	11.17±0.16^{b}
CF	4min	Front	42.21±2.45^{bcd}	1.68±0.32^{cd}	9.29±0.57^{ef}	79.80±1.34^{ab}	9.45±0.61^{efg}
		Back	57.26±0.67^{abc}	1.32±0.03^{bc}	10.42±0.03^{cd}	82.76±0.12^{ab}	10.50±0.03^{cd}
	8min	Front	39.47±1.08^{de}	1.99±0.55^{bc}	8.33±0.27^{fg}	76.67±3.21^{bc}	8.57±0.39^{gh}
		Back	54.91±1.42^{cde}	1.87±0.62^{ab}	10.61±0.17^{bcd}	80.07±3.06^{bc}	10.78±0.27^{bcd}
	12min	Front	38.39±1.29^{e}	2.33±0.19^{ab}	8.31±0.69^{fg}	74.33±0.33^{c}	8.63±0.71^{fgh}
		Back	53.25±2.49^{def}	2.15±0.30^{a}	10.86±0.47^{bc}	78.78±1.55^{c}	11.07±0.47^{bc}
P-value of front			0.000	0.001	0.000	0.000	0.000
P-value of back			0.001	0.000	0.000	0.004	0.000

Means that do not share a letter on superscript in the same column are significantly different. Where, FFPs= fine flour particle size (180µm), MFPs= medium flour particle size (355µm), HFPs=higher flour particle size (500µm) and CFPs= control flour

4.11. Effect of Teff Flour Particle Size and Dough Kneading Time on Proximate Composition of Injera

4.11.1. Moisture Content of Injera

The moisture content of injera ranged from 5.90 to 6.45% on dry base due to the interaction effects of flour particle size and dough kneading time, with significant differences (P<0.05) observed between treatments. The highest moisture value (6.45%) of injera was obtained from control flour kneaded at 12 minute while, the lowest moisture value (5.90%) was observed in injera formulated from coarse flour particle size kneaded for 4 minute. The moisture content of injera made from fine flour particle size and control flour kneaded at all kneading time are not significantly different. The moisture content of injera from medium and coarse flour particle size was not significantly different but significantly different from control flour and fine flour particle size. The results demonstrated that the moisture contents of different injera were significantly influenced by teff flour particle size. Moisture content of injera from fine flour particle size is higher and vice versa.

The difference in moisture content could be due to the low water absorption capacity of medium and coarse flour particle sizes while fine flour particle size and control flour have high water absorption capacity (Y. Assefa *et al*., 2018). The current findings in line with the findings reported by Y Mihrete & Bultosa (2017) who had recorded the moisture content of injera made from the composite flour of teff, sorghum, and faba bean ranged from 5.55% to 8.25%.

4.11.2. Crude Protein Content of Injera

Proteins are source of essential amino acids which are concentrated in the pericarp, aleurone layer and germ of grain (Melese *et al*., 2022). The interaction effect of flour particle size and dough kneading time on the crude protein contents of injera was significant (P<0.05). The values ranged between 10.32 to 12.82%. The control flour particle size injera kneaded for 12 minutes had the highest protein content (12.82%), whereas the fine flour particle size injera kneaded for 4 minutes had the lowest protein content (10.32%). The difference might be attributed due to the difference in flour protein content and dough kneading time which enhances fermentation to increase protein content of injera. Protein content of wheat bread made from fine and coarse flour particle size with dough kneaded for 10 and 12 minute was reported different values by Yaregal et al., (2022). Accordingly 11.23% and 11.88% was recorded when dough was kneaded for 10 and 12 minute, respectively and 10.9% and 11.08% of protein was reported on bread from coarse and fine flour particle size.

The protein content of injera was significantly (P<0.05) affected by dough kneading time. As the dough kneading time increased from 4 minute to 12 minutes the protein content of treatments increased by 13.28% to 23.15%. The current findings agree with the results of Mihrete & Bultosa, (2017), who reported the protein content of fermented injera altered slightly as fermentation time increased. Thus, extended kneading time initiates the fermentation process, which contributes to the increased protein content of the developed injera. This could be due to microbial protein production from metabolic intermediates during their growth cycles. According to Abebe et al., (2015), extending the fermentation time enhanced the crude protein.

4.11.3. Crude Fat Content of Injera

Table 4.10 shows the crude fat content of injera from different teff flour particle size and varied dough kneading time that ranged from 1.18-1.73%. The highest crude fat content (1.73%) was obtained from injera prepared from fine flour particle size and 8 minutes kneading time, while the lowest value (1.18%) was obtained from injera made with coarse flour particle size and 4 minute dough kneading time. The interaction of flour particle size and dough kneading time had a significant (P<0.05) effect on the crude fat content of the Injera. Fine flour particle size and control flour had more germ part of grain that holds more healthy fat content. The current results in line with Fikre, et al., (2019) who reported the fat content from 0.74-2.7%.

Dough kneading traps more air and provides heat for fermentative bacteria and yeast, which initiates dough fermentation. Significant change in dough kneading time demonstrates as fermentation initiated and significantly (P<0.05) reduced the crude fat content of the injera. The fat level of the injeras was shown to be lower under favorable fermenting conditions. This drop in fat content could be related to increased lipolytic enzyme activity during fermentation, which hydrolyzes lipid components into fatty acid and glycerol (Bello *et al.*, 2020). When compared to other staple foods, teff has favorable fatty acid content that dietary fats serve in increasing palatability of food by absorbing and retaining flavours (Mezgebo *et al*., 2018).

4.11.4. Crude Fiber Content of Injera

The crude fiber determines the amount of indigestible cellulose, pentosanes, lignin, and other constituents. Fiber is well recognized for its ability to lower the risk of diabetes, cardiovascular disease, and high blood cholesterol (Memon *et al*., 2020). The crude fiber content of injera was significantly affected ($P<0.05$) by the interactions between flour particle size and dough kneading time and the values were ranged from 2.01 to 3.37%.
The fine flour injera kneaded for 12 minutes yielded the lowest results, and the injera made from coarse flour kneaded for 4 minutes obtained the highest values. The crude fiber values were linearly increasing with the increasing flour particle size and inversely decreased with increase in the dough kneading time. The high content of bran in the coarse particle size flour increase the fiber content of injera made it.

The result also revealed that long kneading time reduced the fiber content of the Injera. The expected decrease in fiber content might be attributed due to favorable condition created for fermentation that help to the partial solubilisation of cellulose and hemi cellulosic type of material by microbial enzymes (Y Mihrete & Bultosa, 2017). The results of the current study were in agreement with the finding of Fikre et al., (2019), who had reported the crude fiber content of injera prepared from teff flour as 3.5%.

4.11.5. Total Ash Content of Injera

The ash content indicates an estimation of the total mineral content in a certain amount of food Substance (Mishra & Chandra, 2012). The total ash contents of injera samples varied significantly ($P<0.05$) due to the interaction of flour particle size and dough kneading time. The ash content of the injera was in the range of 2.40-2.73% (Table 4.10). Injera prepared from coarse flour particle size and kneaded for 4 minutes had the highest total ash (2.73%) value, whereas injera made with fine flour particle size kneaded for 12 minutes had the lowest total ash (2.40%) value. The difference might be due to the flour nature that coarse particle size flour had bran that holds the total mineral content than fine particle size flour

(Y. L. Assefa *et al.*, 2018). The total ash content of injera was not significantly affected by dough kneading time.

4.11.6. Carbohydrate Content of Injera

The interaction effect of flour particle size and dough kneading time had significant effect ($P<0.05$) on the utilizable carbohydrate contents of injera as shown in Table 4.10. The carbohydrate content of the formulated Injera varied between 73.68 and 77.66%. The highest carbohydrate content (77.66%) was found in injera made from fine flour particle size with 4 minute dough kneading time, while the lowest carbohydrate content (73.68%) was found in injera made from control flour and kneaded for 12 minutes. The results revealed that lowering flour particle size increase the carbohydrate content of the injera. This might be due to the difference in starch content of the flour. Teff is starchy cereal that was highly grounded to fine particle size (Baye, 2018). A similar result was reported by Woldemariam et al., (2019), who found the carbohydrate content of amaranthus-Teff-Barley blended injera in the range of 73.89% to 79.71%. The carbohydrate content of injera prepared from medium and coarse flour particle size with all used kneading time was not significantly different.

4.11.7. Gross Energy

The total energy content reflects the presence of carbohydrate, protein, and fat in the diet. The gross energy of injera samples varied from 356.81 to 364.25 kcal/100 g (Table 4.10). A significant difference ($p<0.05$) was observed in the gross energy content of the injera sample by flour particle size and dough kneading time interactions. The highest gross energy content (364.25 kcal/100 g) was observed in injera samples prepared from a fine flour particle size kneaded for 4 minute whereas the lowest energy content (356.81 kcal/100 g) was observed in injera samples prepared from control flour and kneaded to 12 minutes. Injera samples with higher gross energy content indicate the samples attributed to high fat content.

Table 4.10. Effect of flour particle size and dough kneading time on the proximate composition of injera

particle size(μm)	Kneading time(min)	Moisture (% db)	Crude protein (db%)	Crude fat(db%)	Crude fiber (db%)	Total ash (db%)	Carbohydrate (%db)	Gross Energy (kcal/100g)
FFPs	4	6.22±0.06^{abc}	10.32±0.26^{c}	1.53±0.09^{bc}	2.42±0.01^{f}	2.42±0.03^{de}	77.66±0.20^{a}	364.25±0.89^{a}
	8	6.32 ±0.01^{ab}	10.58±0.03^{bc}	1.73±0.05^{a}	2.25±0.05^{g}	2.41±0.03^{e}	77.26±0.01^{ab}	361.99±0.32^{bc}
	12	6.44±0.03^{a}	11.69±0.95^{ab}	1.51±0.04^{bc}	2.01±0.03^{h}	2.40±0.03^{e}	75.86±1.11^{c}	361.02±0.43^{cd}
MFPs	4	5.94±0.24^{d}	10.96±0.11^{bc}	1.37±0.13^{cd}	3.30±0.08^{abc}	2.59±0.06^{bc}	75.92±0.12^{c}	361.28±0.74^{cd}
	8	5.98±0.63^{d}	11.11±0.01^{bc}	1.28±0.01^{d}	3.27±0.01^{abc}	2.60±0.01^{bc}	75.86±0.13^{c}	363.43±0.86^{ab}
	12	6.06±0.28^{cd}	11.71±0.002^{ab}	1.31±0.05^{d}	3.07±0.01^{e}	2.50±0.02^{cd}	74.92±0.16^{cd}	360.08±0.29^{def}
HFPs	4	5.90±1.88^{de}	10.66±0.002^{bc}	1.18±0.05^{d}	3.37±0.02^{a}	2.73±0.01^{a}	76.07±0.00^{bc}	358.43±0.10^{gh}
	8	6.10±0.91^{bcd}	10.66±0.09^{bc}	1.20±0.12^{d}	3.34±0.05^{ab}	2.68±0.0.01^{ab}	76.06±0.01^{bc}	358.67±0.72^{fgh}
	12	6.11±0.71^{bcd}	10.57±0.11^{bc}	1.23±0.05^{d}	3.20±0.07^{cd}	2.68±0.03^{ab}	76.05±0.20^{bc}	357.54±0.09^{hi}
CF	4	6.33±0.19^{ab}	10.41±0.08^{c}	1.60±0.03^{ab}	3.23±0.02^{bcd}	2.52±0.01^{c}	76.09±0.06^{bc}	360.37±0.18^{de}
	8	6.44±0.44^{a}	10.70±0.21^{bc}	1.55±0.01^{abc}	3.12±0.04^{de}	2.52±0.01^{c}	75.65±0.27^{c}	359.37±0.34^{efg}
	12	6.45±0.17^{a}	12.82±0.93^{a}	1.20±0.02^{d}	3.06±0.02^{e}	2.62±0.06^{b}	73.68±0.95^{d}	356.81±0.12^{i}
P-value		0.002	0.000	0.000	0.000	0.000	0.003	0.000

Means that do not share a letter on superscript in the same column are significantly ($p<0.05$) different. Where, FFPs= fine flour particle size (180µm), MFPs= medium flour particle size (355µm), HFPs=coarse flour particle size (500µm) and CF= control flour respectively

4.12. Main Effects of Flour Particle Size and Dough Kneading Time on Mineral Contents of Injera

In this study iron, zinc and calcium contents of injera samples were analyzed and the results are shown in table 4.11. Flour particle size and dough kneading time had significant effect (P<0.05) on the mineral contents of injera. The injera samples made from control flour had the highest values 17.42, 2.33 and 164.89, for iron, zinc and calcium, respectively. Injera samples made from fine flour particle size had lower values of 13.60, 1.85 and 134.14 for iron, zinc and calcium content, respectively. As the dough kneading time increase from 4 minute to 12 minute the mineral content increased from 15.41 to 16.30, 1.95 to 2.25 and 150.53 to 157.00 for iron, zinc and calcium, respectively.

Table 4.11. Main effect of teff flour particle size and dough kneading time on mineral contents of injera.

Particle size	Iron (Fe)	Calcium (Ca)	Zinc (Zn)
FFPs	13.60±0.18^{d}	134.14±3.30^{c}	1.85±0.11^{d}
MFPs	15.75±0.25^{c}	152.17±1.12^{b}	2.05±0.08^{c}
HFPs	16.93±0.09^{b}	162.65±0.55^{a}	2.18±0.05^{b}
CFPs	17.42±0.21^{a}	164.89±0.86^{a}	2.33±0.07^{a}
P-Value	0.000	0.000	0.000
Dough Kneading time			
4 min	15.41±0.35c	150.53±2.05^{c}	1.95±0.08^{c}
8 min	16.05±0.08b	154.37±0.25^{b}	2.12±0.05^{b}
12 min	16.30±0.21a	157.00±0.28^{a}	2.25±0.08^{a}
P-value	0.000	0.000	0.000

Means that do not share a letter on superscript in the same column are significantly different. Where FFps=fine flour particle size (0-180µm), MFPs= medium flour particle size (181-355µm), HFPs= coarse flour particle size (356-500µm) and CFPs= control flour (0-710µm).

4.12.1. Iron Content of Injera

Figure 4.6 shows the iron content of injera samples produced from various teff flour particle sizes and dough kneading times. Interaction effect of flour particle size and dough kneading time had a significant effect (P<0.05) on the iron content of injera. The highest iron content (17.73mg/100g) was obtained in injera samples prepared from control flour kneaded for 8 minute while the lowest iron content (12.87mg/100g) was obtained on injera samples made from fine flour particle size kneaded at 4 minute. The results showed that the particle size of the flour has a positive impact on the iron content of the injera. The iron content of the injera had risen significantly (P<0.05) during dough kneading time. This might be due to long kneading time that initiate the fermentation and enhance the removal of ant-nutritional factors, which are thought to be responsible for protein and mineral unavailability (Yimer Mihrete, 2019).

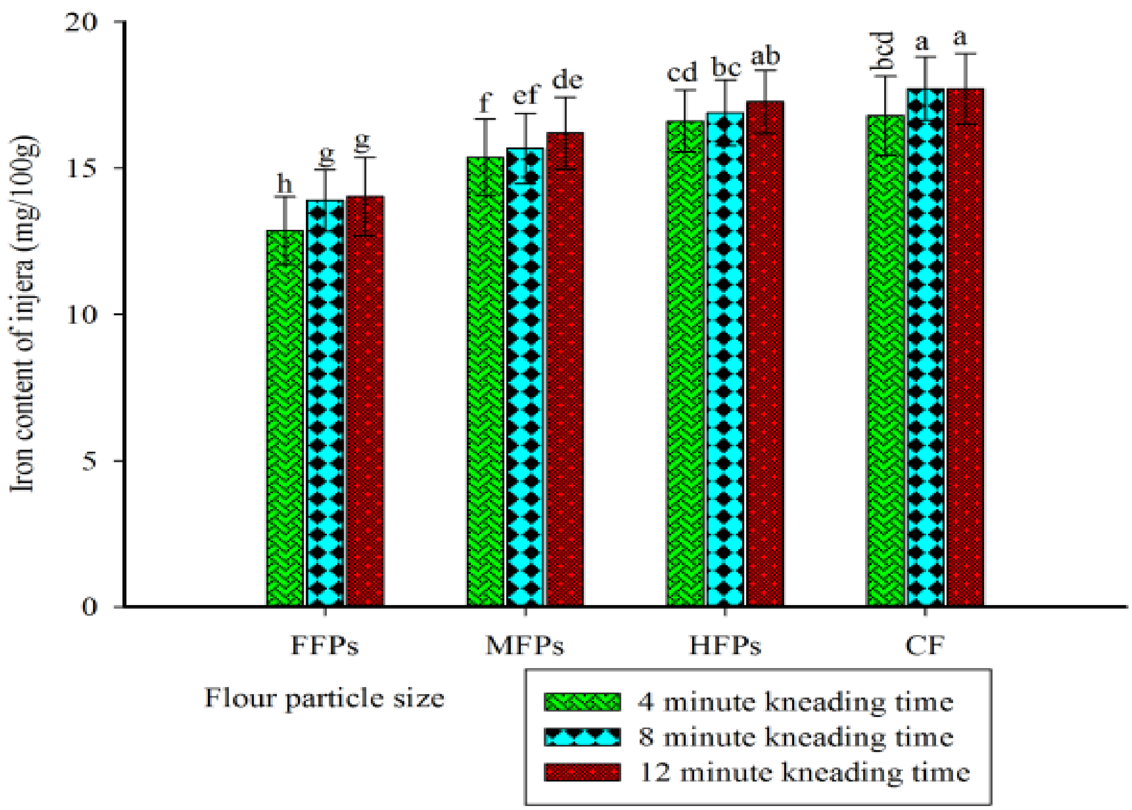


Figure 4.6. Interaction effects of flour particle size and dough kneading time on iron content of injera.

4.12.2. Zinc Content of Injera

Flour particle size and dough kneading time had shown significant effect ($P<0.05$) on zinc contents of injera samples and the results varied between 1.62 – 2.50 mg/100g. The highest value was recorded on injera samples prepared from coarse flour particle size kneaded for 12 minutes whereas the lowest value was found in injera sample prepared from fine flour particle size kneaded for 4 minutes. It was observed that zinc contents increased with increase in kneading time of dough. This is due to the reduction of anti-nutritional factors present in the raw flour. Zinc contents of 3.03 mg/100g, 2.10 mg/100g and 1.44mg/100g were reported by Yasin, (2021), Cherie et al., (2018) and Fikre, et al., (2019), respectively for injera samples prepared from white teff flour. The current study was in the range of previous studies.

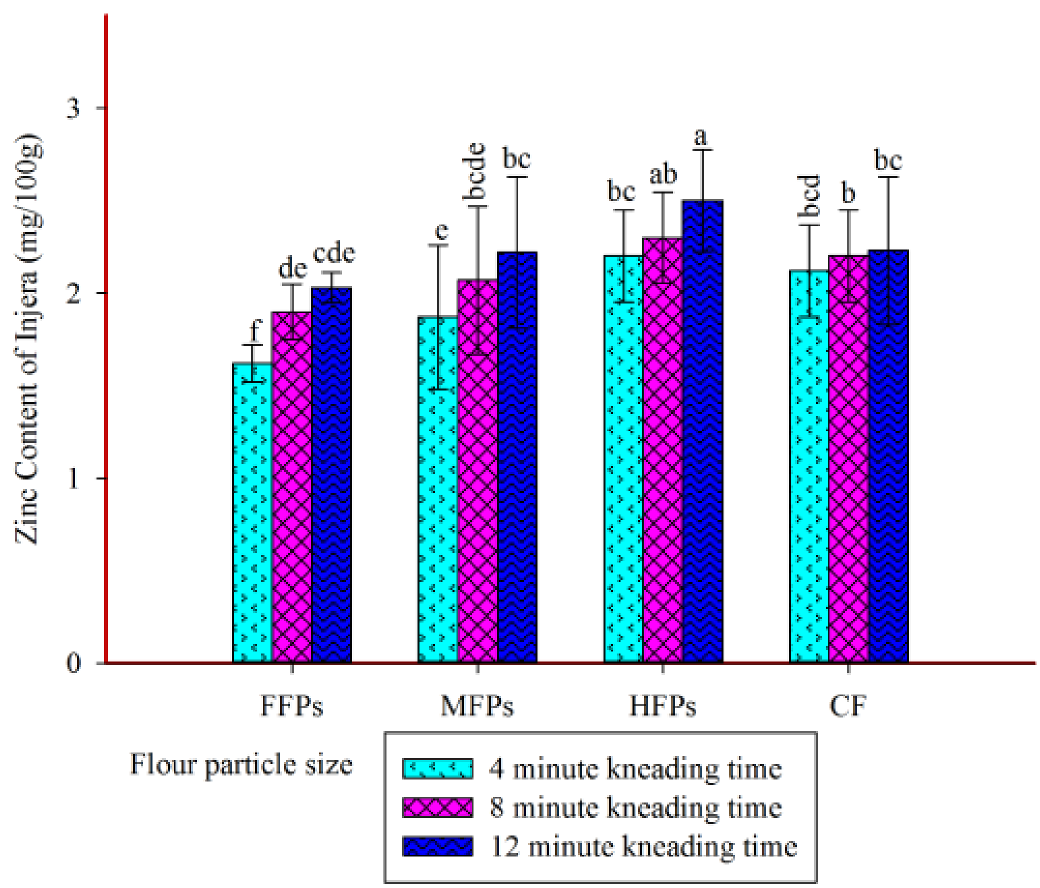


Figure 4.7. Effect of flour particle size and dough kneading time on zinc content of injera.

4.12.3. Calcium Content of Injera

The calcium contents of injera were significantly influenced ($P<0.05$) by the flour particle size and dough kneading time as illustrated on figure 4.8. The values measured ranged from 131.70 mg/100g to 165.95 mg/100g. The maximum (165.95 mg/100g) value was obtained for injera samples prepared from coarse flour particle size and kneaded for 12 minutes, while the lowest (131.70 mg/100g) value was obtained for injera samples prepared from fine flour particle size and 4 minutes kneading time. The calcium content of injera had been found in the ranges of 123-187.25 mg/100g reported previously by Yimer Mihrete (2019). Yegrem & Temesgen, (2019) reported the calcium content of teff injera as 167.69 mg/100g which agree with current findings. Coarse flour particle size had higher calcium content followed by control flour injera than medium and fine flour particle size. The results reveal that reduction in flour particle size decrease the calcium content.

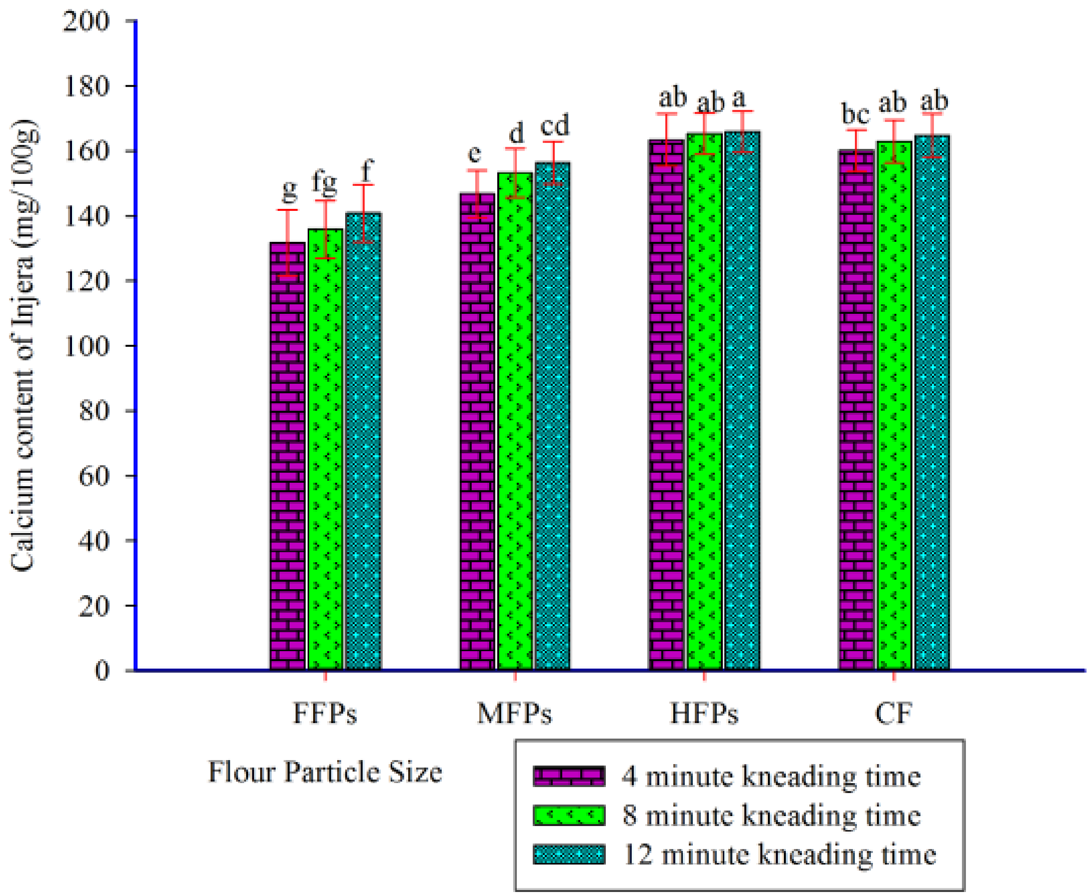


Figure 4.8. Effect of flour particle size and kneading time on the calcium content of injera

4.13. Total Phenolic Compounds and Anti-nutritional Contents of Injera

Table 4.12. Main effect of flour particle size and dough kneading time on total phenolic compounds and anti-nutritional contents of injera.

Flour Particle Size	Total Phenol (mg GAE/100g)	Condensed Tannin (mg catechin equiv./100g)	Phytates (mg/100g)
FFPs	116.61±0.11^{d}	2.69±0.42^{c}	214.77±5.28^{d}
MFPs	121.87±0.62^{c}	3.51±0.43^{b}	239.25±5.97^{c}
HFPs	128.79±1.86^{a}	5.00±0.75^{a}	317.26±1.76^{a}
CF	124.59±2.11^{b}	3.85±0.47^{b}	257.49±1.04^{b}
P-Value	0.000	0.000	0.000
Dough kneading time			
4 min	121.51±0.13^{b}	4.30±1.10^{a}	290.30±0.17^{a}
8 min	122.85±2.74^{ab}	3.70±0.80^{b}	243.07±1.54^{b}
12 min	124.54±3.47^{a}	3.28±0.81^{c}	238.22±1.40^{c}
P-value	0.001	0.000	0.000

Means that do not share a letter on superscript in the same n are significantly different. Where FFps=fine flour particle size (180µm), MFPs= medium flour particle size (355µm), HFPs= coarse flour particle size (500µm) and CF= control flour

4.13.1. Total Phenolic Compounds of Injera

Figure 4.9 presents the impacts of flour particle size and dough kneading time on the total phenol content of injera samples. The interaction of flour particle size and dough kneading time showed significant ($P<0.05$) difference on the overall phenolic content of injera, with values ranging from 114.86 mg GAE/100g to 130.59 mg GAE/100g. The highest value (130.59 mg GAE/100g) was observed in coarse flour particle size injera kneaded for 12 minutes, while the lowest value (114.86 mg GAE/100g) was observed in fine flour particle size kneaded for 4 minutes. Higher total phenolic compounds were obtained in the coarser particle size flour injera as compared to the fine particle size flour injera. The lowest quantity of total phenolic acids indicating that fine particle size fractions primarily consisted of endosperm fraction as higher levels of phenolic acids are associated with the bran fraction than endosperm. The coarse flour particle size was enrichment of bran and germ that was high in total phenolic content (Lu & Luthria, 2016).

The total phenolic content increased with increasing dough kneading time. This might be due to bounded phenolic compounds released with elongated kneading time as a result of heat induced due to friction during kneading (Yoseph, *et al.*, 2018). Through their antioxidant activity, foods high in phenolics may help decrease the chance of strokes, coronary heart disease, certain cancers, and liver disorders (Bhuyan & Basu, 2017).

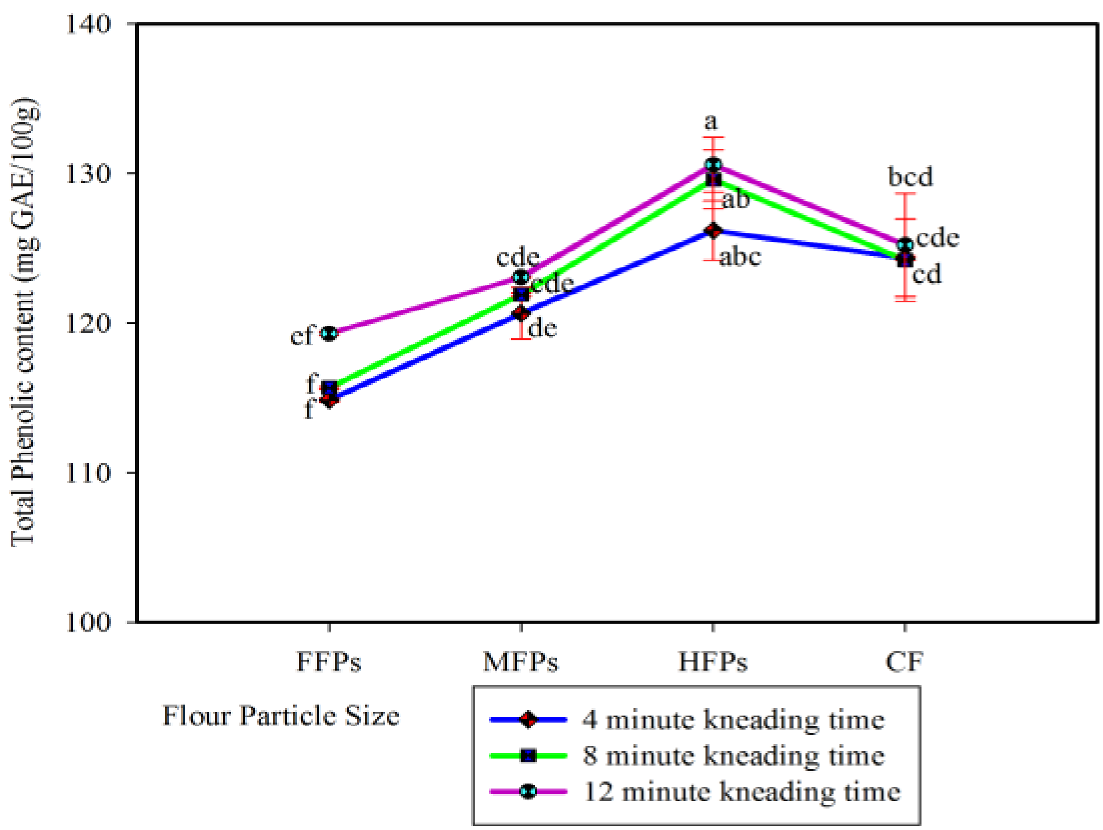


Figure 4.9. Effect of flour particle size and dough kneading time on total phenolic content of injera.

4.13.2. Condensed Tannin Content of Injera

Tannin content of injera ranged from 2.26 mg catechin equiv./100g to 5.90 mg catechin equiv./100g that was significantly ($P<0.05$) affected by flour particle size and dough kneading time. The highest tannin content (5.90 mg catechin equiv./100g) was obtained in the injera samples of coarse flour particle size kneaded for 4 minute and the lowest (2.26 mg catechin equiv./100g) value was obtained on fine flour particle size kneaded for 12 minute. The result indicates that condensed tannin content of the injera samples decreased with elongated dough kneading time, whereas coarse flour particle size injera had greater tannin content than fine flour particle size injera. The higher tannin content in the coarse flour particle size over fine, medium flour particle size and control flour injera might be due to bran amount found in coarse flour. Tannins are known to be accumulated in outer layers of grains that decrease the protein quality of foods and interfere with dietary iron and calcium absorption (Serna-Saldivar & Espinosa-Ramírez, 2019).

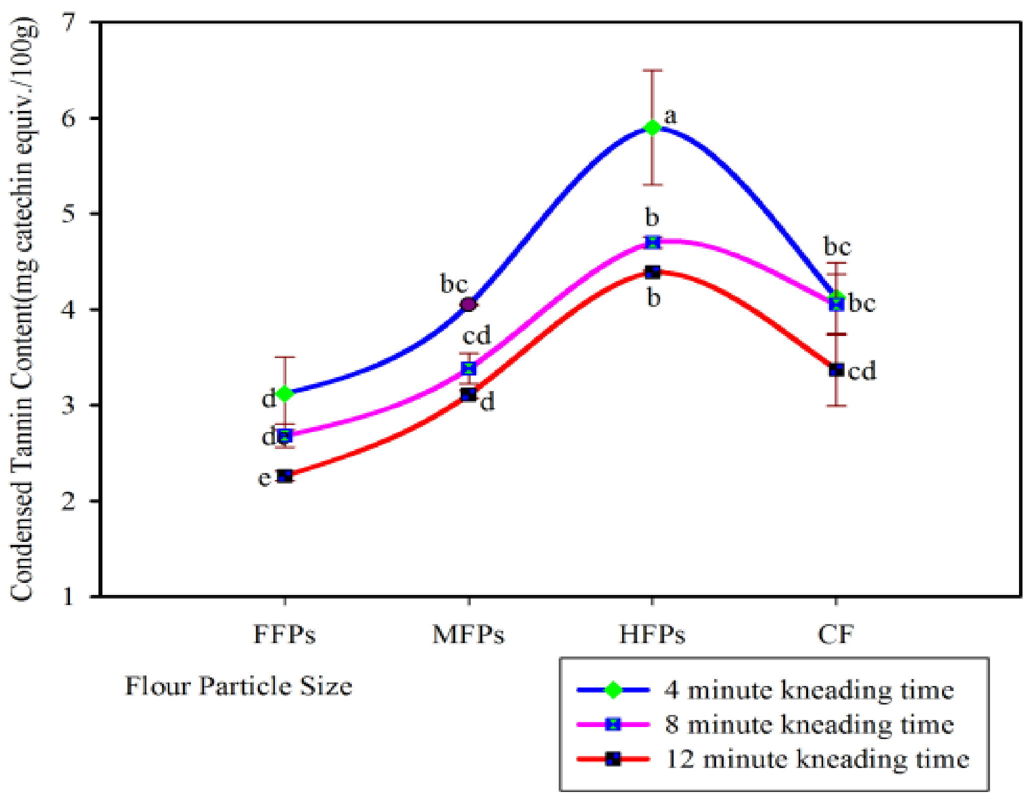


Figure 4.10. Interaction Effect of Flour Particle size and Dough Kneading time on Condensed tannin content of injera.

The tannin content of various sorghum varieties was decreased by 61.9-92.3% after the seed coat/husk was removed. This is due to the presence of phenolic chemicals (tannins) in the pericarp but not in the germ or endosperm (Abiye, 2011). The findings of the current study denote that tannin content of prepared injera was decreased by long time dough kneading. Long time kneading of dough will initiate enzyme activities and fermentation by introducing air and heat for the dough. This activity helps to losses the tannin content of food due to active oxidative enzymes and degradable of pericarp part of cereals with fermentation (Chlopicka *et al*., 2012). On the other hand, the condensed tannin contents of injera samples were 2.69 mg/100g, 3.51mg/100g, 5.00mg/100g and 3.85mg/100g that was lower than 9.30mg/100g, 10.58mg/100g, 12.98mg/100g and 11.19mg/100g found in the flour of fine, medium, coarse particle size and control flour respectively. This could be due to tannin's heat sensitivity and thermal degradation during baking (Yegrem & Temesgen, 2019).

4.13.3 Phytate Content of Injera

Phytic acid is an anti-nutritional component that interacts with carbohydrates, protein, mineral contents like iron, calcium and zinc to produce insoluble complexes that lower their bioavailability, functioning, absorption and nutritional value (Bassi *et al*., 2021). It is the main storage of phosphorus compound of grains. The interaction of flour particle size and dough kneading time had shown a significant ($p<0.05$) effect on the phytate content of injera samples and the values was ranged from 211.02 mg/100g to 329.50 mg/100g. The highest value (329.50 mg/100g) was obtained for coarse flour particle size injera kneaded for 4 minute while the lowest value (211.02mg/100g) was obtained for fine flour particle size injera with 12 minute dough kneading. The phytate content of injera was reduced up to 36% when flour particle size reduced from 500μm to 180μm with kneading time elongated from 4 minute to 12 minute.

The Phytate content of injera samples degraded significantly as the dough kneading time increased. Different content of phytate may come from the influence of kneading which can affect dough fermentation (Urga & Narasimha, H. V, 2017). It decreased in the order kneading time for 4 minute (290.30mg/100g) greater than Kneading for 8 minute (243.07mg/100g) greater than Kneading for 12 minute (238.22mg/100g). Phytate can be degraded by endogenous phytases which can be activated by food processing techniques(Baye *et al*., 2013). Phytates can create complexes with endogenously secreted minerals like calcium and zinc, rendering them unavailable for re-absorption into the body. Increased kneading time can degrade phytate content from food and reduce these impacts (Manary *et al*., 2002). Contrary phytate can help to avoid kidney stones by acting as a crystallization inhibitor of calcium salt. They also have anti-cancer and glucose-lowering qualities (Lee *et al*., 2006).

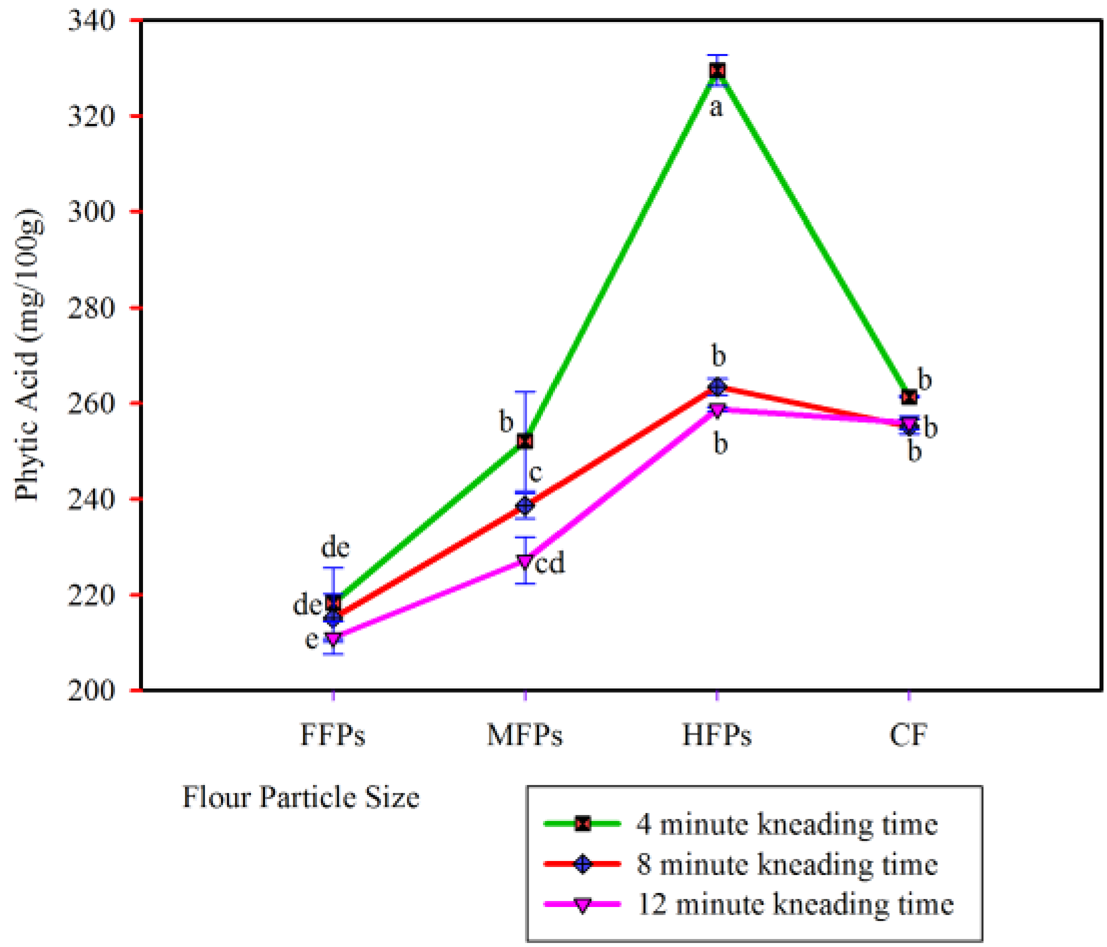


Figure 4.11. Interaction effect of Flour particle Size and Dough Kneading Time on Phytates contents of injera

4.14. Effect of Flour Particle Size and Dough Kneading Time on Microbial Loads of Injera

Figure 4.12 and 4.13 shows the microbial load of injera due to the interaction effect of flour particle size and dough kneading time for day one, day three and day five storage. Figure 4.12 shows the aerobic bacterial count and figure 4.13 shows the yeast-mold counts of injera. The total aerobic bacterial count were not detected in injera samples made from fine and control flour with 4 minute dough kneading time and medium and coarse flour particle size with 4 and 8 minute dough kneading time on day 1 storage while detected up to 4.54 logs CFU/g with increased storage day to 5. The yeast-mold counts of injera samples were found from 1.77-5.08 CFU/g for the analysis conducted in the day one storage, third day and fifth day storage respectively. According to Saddozai & Samina, (2009);(Kasaye & Jha, 2015) based on the WHO regulation of 1994 the standard maximum permissible limits in ready-to-eat baked products (cake, bread, and biscuit) for total aerobic bacterial colony count (total aerobic mesophilic bacterial) are $2.0x10^5$ cfu/g (5.3), coliforms bacteria is 20 cfu/g (1.30), and yeast and mold are 1.0 $x10^4$ cfu/g(4), respectively. Yeasts-molds counts shall not exceed the limits of $1.8x10^3$(3.26) as Ethiopian standard 2013. Compared to standard maximum permissible limits in ready-to-eat baked products, the developed injera from flour particle size and dough kneading time had a lower total plate count profile in all three storage days with yeast-mold load in day one and day three. However, the yeast and mold load of injera on the fifth day of storage exceeds the maximum allowable limits. As a result, the current study suggests that using injera made from different particle sizes with different dough kneading times is safe up to day five storage for bacteria and day three for yeast and mold load. Mold spoilage is a serious issue that decreases food's shelf life (Gill *et al*., 2020). Overall, under traditional storage conditions, injera storage does not usually last more than three days at room temperature due to mold spoilage (Ashagrie & Abate, 2012).

4.14.1. Total Plate Count of Injera

The total aerobic bacteria load of injera prepared from different particle size and varied mechanical dough kneading time is presented in figure 4.12 and appendix table 3. As explained in appendix table 3 at 1^{st} day no bacteria colony count was observed on injera made from fine flour kneaded for 4 minute, medium flour for 4 minute, medium for 8 minute, coarse for 4 and 8 minute, and control flour for 4 minute kneading time. The interaction effect of the main factors had no significant effect on the total aerobic plate counts of injera samples of fine flour with 8 minute kneading time, fine flour with 12 minute dough kneading time, medium flour with 12 minute, coarse flour with 12 minute, control flour with 8 minute and control flour with 12 minute dough kneading time on the day one storage.

The interactions of flour particle size and dough kneading time resulted a total aerobic plate counts ranged from 3.49 – 3.77 log CFU/g (day three) and 3.97 – 4.54 log CFU/g (day five). At 3rd day of storage the highest bacterial colonies count was obtained in injera samples formulated from fine flour particle size and 12 minute dough kneading time and control flour with 12 minute dough kneading time having the values of 3.75 and 3.77 respectively while the lowest bacterial colonies count was obtained in samples of medium flour kneaded for 8 minute followed by medium flour kneaded at 4 minute and coarse flour kneaded at 4 minute with the values of 3.49 and 3.52 logs CFU/g respectively. The result was in close agreement to total aerobic count of injera with the value of 3.07 log CFU/g after 4 days and that rose to 3.89 log CFU/g after 6 days storage reported by Kelbore et al., (2022). This might be due to favorable condition (air and temperature) formed during mixing and kneading for microbial growth (Eke & Elechi, 2021).

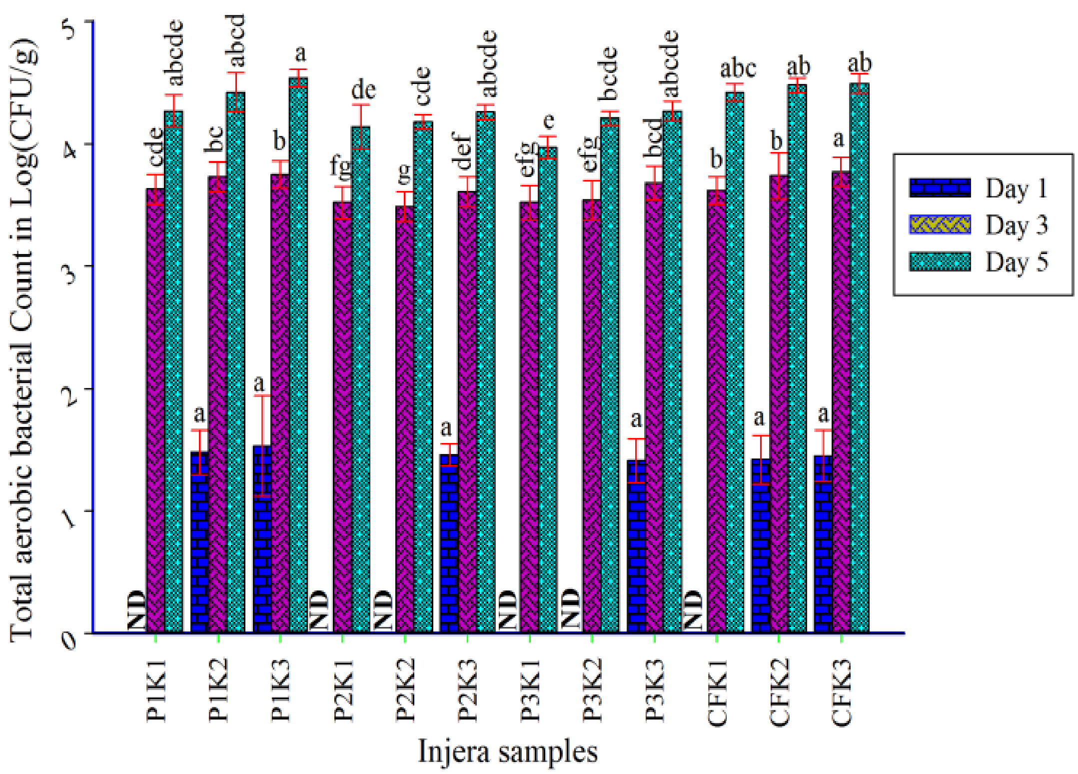


Figure 4.12. Total aerobic bacteria count of injera on day 1, day 3 and day 5 storage.

Where: P1K1 is fine flour particle size with 4 minutes of dough kneading time, P1K2 is Fine flour particle size with 8 minutes of dough kneading time, P1K3 is Fine flour particle size with 12 minutes of dough kneading time, P2K1 is medium flour particle size with 4 minutes of dough kneading time, P2K2 is medium flour particle size with 8 minutes of dough kneading time, P2K3 is medium flour particle size with 12 minutes of dough kneading time and P3K1 is coarse flour particle size with 4 minutes of dough kneading time, P3K2 is coarse flour particle size with 8 minutes of dough kneading time, P3K3 is coarse flour particle size with 12 minutes of dough kneading time, CFK1 is control flour with 4 minute dough kneading time, CFK2 is control flour with 8 minute dough kneading time and CFK3 is control flour with 12 minute dough kneading time, ND is Not Detected.

Different flour particle size with increased dough kneading time initiate the fermentation that promotes growth of bacterial load with maximum kneading time of injera dough (Godebo *et al*., 2019). On the other hand the difference in nutritional content, the moisture holding capacity of varied flour particle size might affect the microbial load of injera prepared from different flour particle size with varied dough kneading time (Ijah et al., 2014). When compared to control and fine flour particle size injera, injera made from medium and coarse flour particle size was slightly lower. This is due to the difference in antimicrobial compounds, such as phenolic compounds, which was higher in medium and coarse flour (Girma *et al*., 2013a)

4.14.2. Mold and Yeast Counts in Injera

The yeast-mould counts were significantly different ($P<0.05$) for flour particle size and dough kneading time differences in all injera storage days as shown on the figure 4.13. The result of yeast-mould counts of injera samples from interaction effect of flour particle size and dough kneading time ranged from 1.77-2.09 log CFU/g on day one storage, 3.76- 4.29 log CFU/g on day three storage and lastly rose to 4.48-5.08 on day 5 storage. The highest score of total yeast and mold counts were obtained in injera prepared with fine flour particle size and 12 minute kneading time and control injera with 12 minute kneading time. The lowest values of total yeast-mold counts were recorded in the injera sample prepared from coarse flour particle size with 4 minute dough kneading time. The result was higher than the work of Girma et al., (2013) 2.85, 3.06 and 4.08 log CFU/g on day 2, 4 and 6 storage time, respectively. This could be due to the fine flour particle size and control flour injera contained more nutrients, such as proteins and starch, than medium and coarse flour injera, which could be easily available to microorganisms, leading to higher counts (Girma *et al.*, 2013a). The moisture content of injera prepared from fine flour particle size and control flour had higher which is suitable for yeast and mold growth (Ijah *et al.*, 2014). In the current study the higher counts of yeast and mold was higher in fine flour particle size and control flour injera and lower in injera prepared from medium and coarse flour particle size that contained lower protein and starch. The spontaneously kneading injera dough promotes the growth of natural microorganisms by promoting air and temperature and aids in the diffusion of nutrients for stationary yeast and mould (Akdoğan & Özilgen, 1992). The result of current study concludes that increment of dough kneading time increases the growth of microbial load beyond the permissible limit on the day 5 storage.

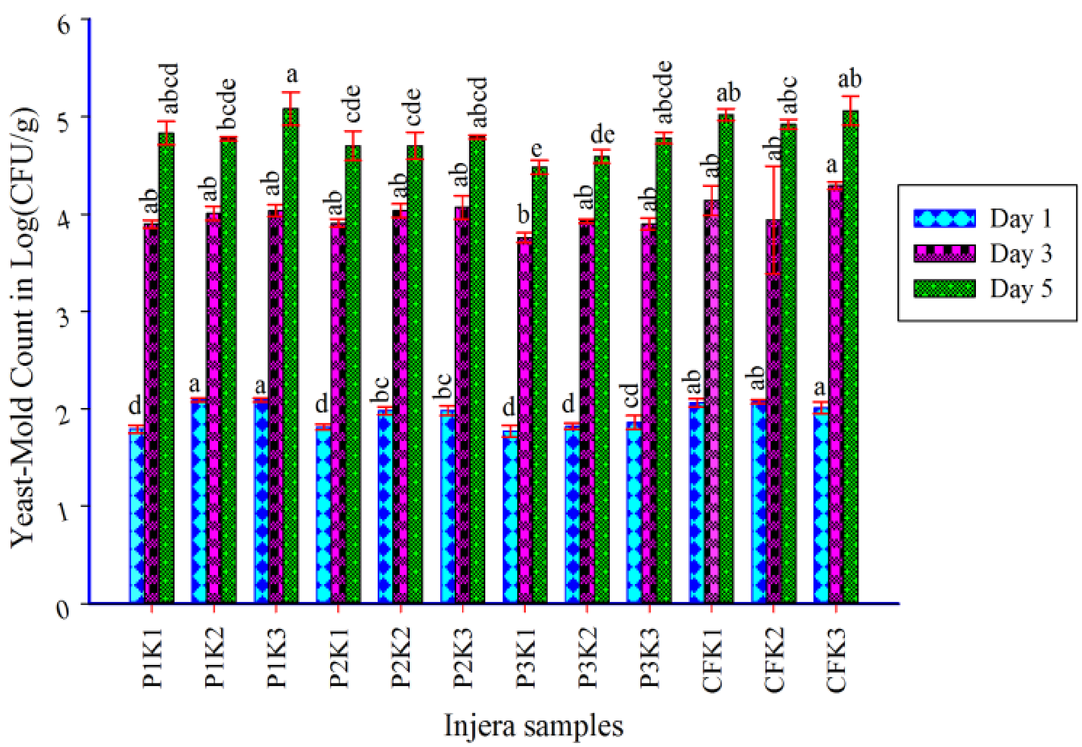


Figure 4.13. Total yeast and mold count of injera on day 1, day 3 and day 5 storage.

Where: P1K1 is fine flour particle size with 4 minutes of dough kneading time, P1K2 is Fine flour particle size with 8 minutes of dough kneading time, P1K3 is Fine flour particle size with 12 minutes of dough kneading time, P2K1 is medium flour particle size with 4 minutes of dough kneading time, P2K2 is medium flour particle size with 8 minutes of dough kneading time, P2K3 is medium flour particle size with 12 minutes of dough kneading time and P3K1 is coarse flour particle size with 4 minutes of dough kneading time, P3K2 is coarse flour particle size with 8 minutes of dough kneading time, P3K3 is coarse flour particle size with 12 minutes of dough kneading time, CFK1 is control flour with 4 minute dough kneading time, CFK2 is control flour with 8 minute dough kneading time and CFK3 is control flour with 12 minute dough kneading time

4.15. Sensory Acceptability of Injera

4.15.1. Main effect of Flour Particle Size and Dough Kneading Time on sensory acceptability of injera

In the current study panelists judge sensory attributes with five point hedonic scale to assess the acceptability of injera. Color, taste, texture, eye size, rollability, eye distribution, top and bottom surfaces, and overall acceptability of injera samples were judged by panelists, as shown in Table 4.13. The taste of injera is attributed with the sweet, sour, and bitter feelings evoked in the mouth by injera contact. One of the most important parameters is the appearance of injera, which refers to the quality of the eyes (cells) of the honeycomb-like structure of the top surface of injera formed during cooking due to escaping CO_2 bubbles (Yimer Mihrete, 2019).

Injera prepared from fine flour particle size and control flour had higher panelist acceptability in all sensory attributes as compared to the corresponding medium and coarse flour particle size. The medium flour particle size and coarse flour particle size injera had significantly different and had lower sensory acceptable. The medium and coarse flour particle size injera`s sensory attributes scores were not good as per the scale as the highest value, 4.52, was below like moderately (4) scale and above the dislike one. The result was mostly fall in the either score scale of like or dislike (3) and below this value for most of sensory attributes of all injera samples of medium and coarse flour particle size. That is to say, if the particle size is coarse the popularity of the injera reduced.

The sensory attributes of injera was varied with the kneading conditions of the dough. This could be explained by the relationship between kneading and gas formation in the dough of injera which results fermentation conditioning. Kneading the dough encourages the release of large gas bubbles, resulting in a more even distribution of the bubbles within the dough, which ultimately contributes to the product's quality (Rosell, 2011). The score of sensory quality of injera increase with the increments of kneading time from 4 minutes to 12 minutes. This result concludes that, dough kneading parameters should always be optimised for each preparations and process, taking into account kneading rate, duration, and stand mixer type (Y.Assefa *et al.*, 2018). When the flour particle size was reduced to less than 180µm, the panelists' scores were like moderately and like very much, and when the dough kneading time was increased from 4 to 12 minutes, the sensory score changed from dislike to like moderately in all sensory attributes. Sensory quality of injera revealed that the appearance (color, eye distribution and eye size) texture, taste, top and bottom surfaces and overall acceptability of the injera prepared from fraction 180mm kneaded with 12 minute dough kneading time got higher scores than the injera prepared from other fractions of flour with lower minute dough kneading time.

Table 4.13. Main effect of flour particle size and dough kneading time on sensory acceptability of injera

Flour particle size	Color	Taste	Eye Size	Rollability	Texture	Eye Distribution	Top and Bottom Surface	OAA
FFPs	4.52±0.75[a]	4.39±0.79[a]	4.41±0.70[a]	4.54±0.72[a]	4.51±0.70[a]	4.41±0.73[a]	4.49±0.67[a]	4.50±0.65[a]
MFPs	2.89±0.89[c]	2.98±0.92[b]	2.80±0.92[b]	3.02±1.04[b]	2.80±0.91[b]	2.84±1.07[b]	2.97±0.87[b]	2.87±0.83[b]
HFPs	2.50±0.92[d]	2.48±0.91[c]	2.13±0.91[c]	2.52±0.90[c]	2.24±0.87[c]	2.22±0.87[c]	2.29±0.86[c]	2.41±0.71[c]
CF	4.24±0.86[b]	4.19±0.76[a]	4.24±0.91[a]	4.36±0.73[a]	4.26±0.78[a]	4.23±0.71[a]	4.30±0.70[a]	4.28±0.61[a]
P-Value	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000
Dough kneading time								
4 min	3.34±0.81[b]	3.41±0.82[b]	3.16±0.86[b]	3.44±0.63[b]	3.32±0.82[b]	3.25±0.60[b]	3.43±0.63[b]	3.38±0.55[b]
8 min	3.49±0.59[b]	3.45±0.83[ab]	3.30±1.12[b]	3.57±1.01[b]	3.38±0.96[b]	3.30±0.97[b]	3.31±0.91[b]	3.38±0.77[b]
12 min	3.79±0.84[a]	3.67±0.71[a]	3.73±0.74[a]	3.82±0.55[a]	3.66±0.84[a]	3.72±0.55[a]	3.79±0.57[a]	3.79±0.50[a]
P-value	0.000	0.012	0.000	0.000	0.004	0.000	0.003	0.000

Values are mean ± SD and values in the same column with different superscript letters are significantly different from each other (P <0.05) Where: FFPs= fine flour particle size (0-180μm), MFPs= medium flour particle size (181-355μm), HFPs= higher/coarse flour particle size (356-500μm), CF= control flour

4.15.2. Interaction Effect of Flour Particle Size and Dough Kneading time on Sensory Acceptability of Injera

The sensory acceptability of injera prepared from different flour particle size and dough kneading time was presented in the figure 4.14. Injera prepared from fine flour particle size and control flour with 12 minute kneading time had higher acceptance by sensory evaluation panelists in all sensorial characteristics. All the sensory attributes of Injera formulated from coarse flour particle size with 4 minutes dough kneading time scored mean of below 2 that is the lowest score from all injera formulations. As kneading time increased to 8 minutes and 12 minutes, respectively; the acceptability of injera in the range of flour particle size from 356µm-500µm is increased to almost 3 mean score on average. This implies kneading time improves the acceptability of injera. The sensory attributes are affected as the flour particle size and kneading time changes. The change of flour particle size is related with functional properties such as water absorption capacity and change of kneading time affects injera due to favorable condition on fermentation that forms gas. Dough kneading helps for the formation of gas during fermentation. Gas bubbles are more likely to be released during kneading or mixing of the dough, which leads to an equal distribution of the bubbles throughout the dough and ultimately improves the final injera quality.

The interaction of flour particle size and dough kneading time had significant ($P<0.05$) effect on the color of injera. The lowest color (1.93±0.96) acceptability score was noted for injera with 500µm flour particle size and 4 minute kneading time while the highest score 4.59±0.59 was for injera prepared from fine particle size with 8 minute dough kneading time. The color acceptability of injera was more dominantly affected by both particle size and dough kneading time. Injera prepared from fine particle size with all stated dough kneading time was higher than that of control injera but not significantly different. But injera prepared from medium and coarse particle size was significantly different from control and fine particle size on the color characteristics.

Similarly the taste score of injera not exhibited significant ($P>0.05$) differences due to the interaction between fine and control flour particle size and given dough kneading time while injera prepared from medium and coarse flour particle size with varied dough kneading time was significantly different from each other and control injera. The scores were more influenced by particle size rather than dough kneading time. The highest score, 4.56±0.71 was for injera prepared from fine (180µm) teff flour and 12 minutes of kneading time and the lowest score, 2.22±0.99, was for injera with coarse (500µm particle size) flour and dough kneading time for 4 minutes. This implies the injera prepared from flour with higher kneading time had more acceptance of taste than other injera prepared from either medium or coarse flour with lower kneading time. Taste refers to the flavors of sweetness, sourness, saltiness, and bitterness that are triggered in the mouth through injera encounter (Ghebrehiwot *et al*., 2016).

An essential sensory characteristic that is frequently used to assess the quality of baked goods made with cereal is texture. It describes the degree of fluffiness, roughness, smoothness, softness or hardness of injera and is assessed by touch. The interaction effect of flour particle size and dough kneading time had no significant ($P>0.05$) effect on the texture of injera prepared from fine and control particle size with all kneading time except injera from control particle size with 4 minute dough kneading time. However flour particle size and dough kneading time had significant ($p<0.05$) effect on the texture of injera prepared from medium, coarse and control with 4 minute dough kneading time. The highest score of texture is 3.93 for injera prepared from control flour particle size with 4 minute dough kneading time and the minimum score is 1.85 for injera prepared from coarse flour particle size with 4 minute dough kneading time. These were all significantly different from other samples including control injera of with 8 and 12 minutes dough kneading time.

The eye uniformity (eye size and eye distribution) of injera differed significantly ($P<0.05$) due to the interaction effect of flour particle size and dough kneading time. The highest scores in the eyes sizes and eye distribution (4.63 and 4.59) were determined in fine flour particle size with 12 minute kneading time and control injera with 8 minute kneading time, respectively. The lowest scores in eye size and eye distribution (1.56 and 1.61) determined in injera prepared from coarse flour particle size with minimum (4 minute) kneading time. As the flour particle size decreased to fine flour (below 0.18mm) the injera was resulting in a few scattered eyes and even eye distribution. On the other hand as the dough kneading time increases from 4 minute to 12 minutes the fermentation increases and bubble gases are formed resulting acceptable eye size and distribution.

The interaction of the two factors on the rollability and top and bottom surface of injeras revealed significant ($P<0.05$) differences among the samples and control samples. The highest score was 4.66 of the samples belonged to fine flour particle size injera with 12 minute dough kneading time and the lowest score was 1.93 and 1.83 with 4 minute dough kneading time for rollability and top and bottom surface respectively. Injera's desirable characteristics include softness, non-stickiness, and rollability without fracture *(*Yetneberk *et al*., 2005).

The aggregate of customer or panelist evaluations of a product is referred to as the product's overall acceptability (Ghebrehiwot *et al*., 2016). In this work, results of the panelist were not significantly different in the overall acceptability of formulations on fine and control flour injera. Injera of fine flour particle size with all given dough kneading time and injera of control flour particle size with 8 and 12 minutes kneading time were 4.29, 4.54, 4.56, 4.56 and 4.66 respectively. The analysis of variance indicated that the quality of injera made from control flour particle size with 8 and 12 minutes of kneading time and injera prepared from 0-180µm particle size of flour with all of the kneading time did not substantially differ between them while different from other formulations of flour particle size. Injera prepared from control and fine flour particle size with 12 minute dough kneading time gained higher score in all sensory attributes. Fine flour particle size with 12 minute dough kneading time had the highest overall acceptability value, followed by injera made from fine flour particle size with 8 minute dough kneading time and injera of control flour with 12 minute dough kneading time, while injera made from coarse flour particle size with 4 minute dough kneading time had the lowest value (1.95).

According to the current results, milling the teff grain to a fine particle size of less than 180µm and kneading the dough for more than 8 minutes significantly increased the sensory score in all quality attributes, thereby increasing the kneading time of coarse particle size flour to 12 minutes increased the rollability, eye distribution, and overall acceptability of injera as compared to the control flour injera.

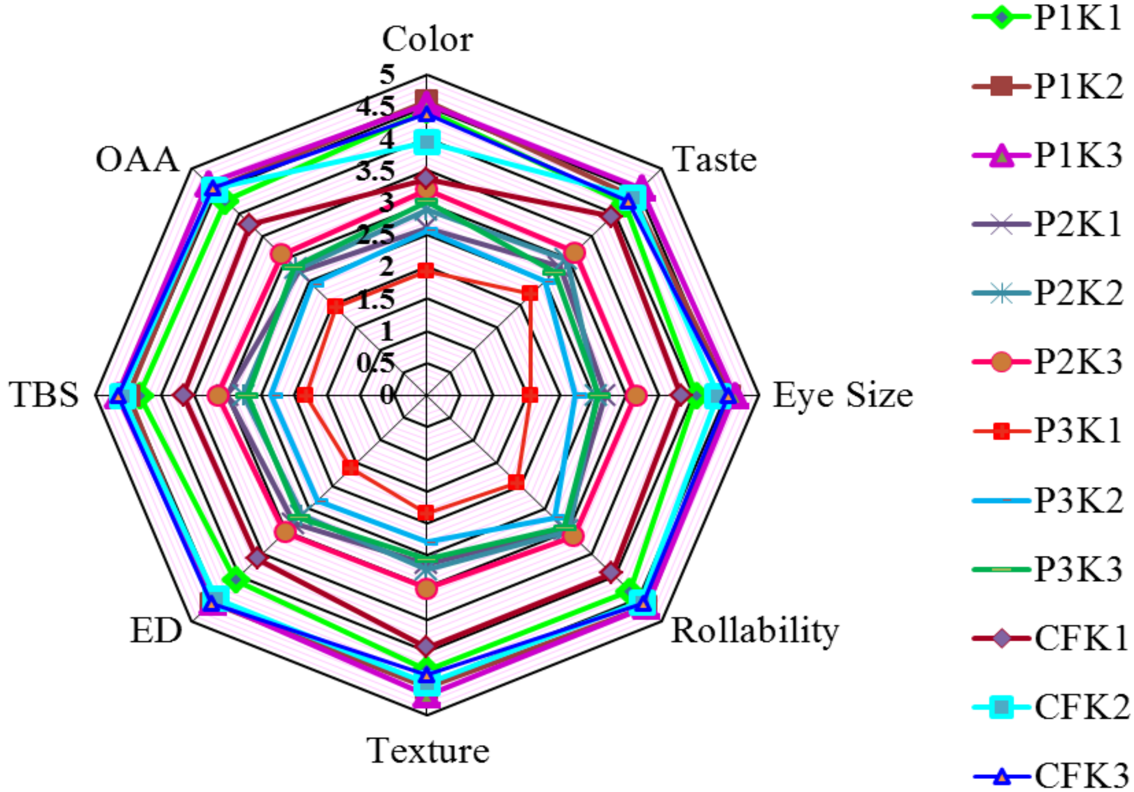


Figure 4.14. Web-chart of eight sensory attributes of injera prepared from different particle size of flour with varied dough kneading time

Where OAA= Overall acceptability, TBS= Top and bottom surface, ED= Eye distribution, Where: P1K1 is fine flour particle size with 4 minutes of dough kneading time, P1K2 is Fine flour particle size with 8 minutes of dough kneading time, P1K3 is Fine flour particle size with 12 minutes of dough kneading time, P2K1 is medium flour particle size with 4 minutes of dough kneading time, P2K2 is medium flour particle size with 8 minutes of dough kneading time, P2K3 is medium flour particle size with 12 minutes of dough kneading time and P3K1 is coarse flour particle size with 4 minutes of dough kneading time, P3K2 is coarse flour particle size with 8 minutes of dough kneading time, P3K3 is coarse flour particle size with 12 minutes of dough kneading time, CFK1 is control flour with 4 minute dough kneading time, CFK2 is control flour with 8 minute dough kneading time and CFK3 is control flour with 12 minute dough kneading time.

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

The current study investigated the effects of flour particle size and dough kneading time on injera's physico-chemical characteristics, dough rheological properties, microbiological quality and sensory acceptability of injera. Milling teff grain and subsequent separation of flours into different particle size ranges had various effects on chemical and physical properties of flours and final products. The interaction of flour particle size and dough kneading time significantly ($p<0.05$) affects the physicochemical, functional, and rheological characteristics of dough, as well as the sensory acceptability and microbial quality of injera.

Fine flour particle size scores the highest percentage of water solubility index and water absorption index, indicating suitability to produce higher quality injera. Fine flour particles contained more protein, starch, and fat, whereas coarse flour particles contained higher ash, mineral, phenolic compounds, and ant-nutritional factors. Coarse flour particle size incorporated more bran (outer layer) of cereal than fine flour, which contained more of endosperm. Dough kneading time considerably affect physico-chemical properties and sensory quality of injera. Mechanical dough kneading for more time promote the product to the overall acceptability on sensory analysis, improve proximate composition and total phenolic compounds of injera, minimize the anti-nutritional factors of injera and enhances the bioavailability by initiating fermentation through air and temperature introducing to dough.

Injera making process requires the improvement of flour particle size and dough kneading time as these affects the dough rheological properties and injera quality. In general, flour particle size distribution to make quality and acceptable injera ranges from 0-180µm with mechanical dough kneading time of 12 minutes. So the findings of this study would contribute for the decision of flour particle size and using of mechanical dough kneader for the development of quality and acceptable injera especially in the industrial injera manufacturing.

5.2. Recommendations

- The current study recommends optimized flour particle size to 180μm and mechanical dough kneading time of 12 minute to maintain high quality and uniformity of injera.
- The commercial teff flour mill can use the method of fractionating flour into different particle sizes by sieving to produce high quality injera.
- The firms that produce injera in mass should include mechanical kneading mechanism to obtain high quality product and ignore fatigue of human on hand kneading.
- Based on the findings of this study of flour particle size and dough kneading time, the amount of absit added to dough of different flour particle size in relation to injera quality is an area that needs to be investigated further.
- Flour particle size is related with the starch properties that determine the final product. In the future, researchers may study damaged starch, amylose content and starch digestibility of different flour particle size on the quality and acceptability of injera.

REFERENCES

Abebaw, G. (2020). The Study of Some Particle Size Distribution of Teff [Eragrostis teff (Zucc.) Trotter] Grain Cultivars and Its Flour. *Journal of Food and Nutrition Sciences*, *8*(4), 108–111. Doi: 10.11648/j.jfns.20200804.16

Abebe, W., Collar, C., & Ronda, F. (2015). Impact of variety type and particle size distribution on starch enzymatic hydrolysis and functional properties of tef flours. *Carbohydrate Polymers*, *115*, 260–268. https://doi.org/10.1016/j.carbpol.2014.08.080

Abebe, W., & Ronda, F. (2014). Rheological and textural properties of tef [Eragrostis tef (Zucc.) Trotter] grain flour gels. *Journal of Cereal Science*, *60*(1), 122–130.

Abebe, W., Ronda, F., Villanueva, M., & Collar, C. (2015). Effect of tef [Eragrostis tef (Zucc.) Trotter] grain flour addition on viscoelastic properties and stickiness of wheat dough matrices and bread loaf volume. *European Food Research and Technology*, *241*(4), 469–478. https://doi.org/10.1007/s00217-015-2476-0

Abewa, A., Adgo, E., Yitaferu, B., Alemayehu, G., Assefa, K., Solomon, J. K. Q., & Payne, W. (2019). Teff grain physical and chemical quality responses to soil physicochemical properties and the environment. *Agronomy*, *9*(6), 283.

Abiye, K. (2011). *Effect of processing on Nutritional and Anti nutritional Compositions, and Functional Properties of Selected Sorghum Varieties Grown in Ethiopia*. Addis Ababa University.

Abraha, A., & Abay, F. (2017). Effect of different cereal blends on the quality of Injera a staple food in the highlands of Ethiopia. *Momona Ethiopian Journal of Science*, *9*(2), 232–241. DOI: 10.4314/mejs.v9i2.7

Achenef, M., Basaznew, B., Mersha, C., Tewodros, F., & Ayalew, B. (2013). Study on utilization and efficacy of commonly used anthelmintics against gastrointestinal nematodes in naturally infected sheep in North Gondar, North-Western Ethiopia. *African Journal of Pharmacy and Pharmacology*, *7*(12), 673–678.

Agza, B., Bekele, R., & Shiferaw, L. (2018). Quinoa (Chenopodium quinoa, Wild.): As a potential ingredient of injera in Ethiopia. *Journal of Cereal Science*, *82*, 170–174.

Ahmed, J., Al-Foudari, M., Al-Salman, F., & Almusallam, A. S. (2014). Effect of particle size and temperature on rheological, thermal, and structural properties of pumpkin flour dispersion. *Journal of Food Engineering*, *124*, 43–53.

https://doi.org/10.1016/j.jfoodeng.2013.09.030

Ahmed, J., Thomas, L., & Arfat, Y. A. (2019). Functional, rheological, microstructural and antioxidant properties of quinoa flour in dispersions as influenced by particle size. *Food Research International*, *116*, 302–311. Doi.org/10.1016/j.foodres.2018.08.039

Akdoğan, H., & Özilgen, M. (1992). Kinetics of microbial growth, gas production, and dough volume increase during leavening. *Enzyme and Microbial Technology*, *14*(2), 141–143. https://doi.org/10.1016/0141-0229(92)90172-K

Alemneh, S. T., Emire, S. A., Hitzmann, B., & Zettel, V. (2022). Comparative Study of Chemical Composition, Pasting, Thermal and Functional properties of Teff (Eragrostis tef) Flours Grown in Ethiopia and South Africa. *International Journal of Food Properties*, *25*(1), 144–158. https://doi.org/10.1080/10942912.2022.2027441

Ali, R., Khan, M. S., Sayeed, S. A., Ahmed, R., Saeed, S. M. G., & Mobin, L. (2014). Relationship of damaged starch with some physicochemical parameters in assessment of wheat flour quality. *Pak. J. Bot*, *46*(6), 2217–2225.

Anberbir, S. M., Satheesh, N., Abera, A. A., Tenagashaw, M. W., Asres, D. T., Tiruneh, A. T., Habtu, T. A., Awol, S. J., & Wudineh, T. A. (2023). Effect of blending ratio and fermentation time on the physicochemical, microbiological, and sensory qualities of injera from teff, pearl millet, and buckwheat flours. *CyTA-Journal of Food*, *21*(1), 217–236. https://doi.org/10.1080/19476337.2023.2188058

Aprianita, A., Purwandari, U., Watson, B., & Vasiljevic, T. (2009). Physico-chemical properties of flours and starches from selected commercial tubers available in Australia. *International Food Research Journal*, *16*(4), 507–520.

Ashagrie, Z., & Abate, D. (2012). Improvement of injera shelf life through the use of chemical preservatives. *African Journal of Food, Agriculture, Nutrition and Development*, *12*(5), 6409–6423. DOI: 10.18697/ajfand.53.10910

Asres, D. T., Nana, A., & Nega, G. (2018). Complementary feeding and effect of spontaneous fermentation on anti-nutritional factors of selected cereal-based complementary foods. BMC Pediatrics, 18(1), 1–9. https://doi.org/10.1186/s12887-018-1369-3

Assefa, Y., Emire, S., Villanueva, M., Abebe, W., & Ronda, F. (2018). Influence of milling type on tef injera quality. *Food Chemistry*, *266*, 155–160.

Assefa, Y. L., Emire, S. A., Abebe Zeleke, W., & Ronda Balbás, M. F. (2018). Effect of mill type and mechanical kneading conditions on fermentation kinetics of tef dough during injera making and phytate to mineral molar ratio of injera. *Research & Reviews: Journal of Food Science and Technology*, *7*(2), 9–19.

Assefa, Y. L., Emire, S. A., Abebe Zeleke, W., Villanueva Barrero, M., & Ronda Balbás, M. F. (2018). The effect of mechanical kneading and Absit preparation difference on tef injera quality. *African Journal of Food Science*, *12*(10), 246–253.

Attuquayefio, W. D. (2014). *Influence of processing parameters on eye size and elasticity of tef-based injera*. https://etda.libraries.psu.edu/files/final_submissions/10331

Attuquayefio, W. D. (2015). *Influence of processing parameters on eye size and elasticity of tef-based injera*. https://etda.libraries.psu.edu/files/final_submissions/10331

Atwell, W., & Finnie, S. (2016). *Wheat flour*. Elsevier.

Authors, T. (2022). *Total phenolics and antioxidant capacity of the white and brown teff [*. *36*(4), 749–766. DOI: 10.4314/bcse.v36i4.3

Awolu, O. O., Oluwaferanmi, P. M., Fafowora, O. I., & Oseyemi, G. F. (2015). Optimization of the extrusion process for the production of ready-to-eat snack from rice, cassava and kersting's groundnut composite flours. *LWT-Food Science and Technology*, *64*(1), 18–24. https://doi.org/10.1016/j.lwt.2015.05.025

Awuchi, C.-G., Igwe, V.-S., & Echeta, C.-K. (2019). The functional properties of foods and flours. *International Journal of Advanced Academic Research*, *5*(11), 139–160.

Ayo-Omogie, H. N., & Ogunsakin, R. (2013). *Assessment of chemical, rheological and sensory properties of fermented maize-cardaba banana complementary food*.

Bala, M., Handa, S., Mridula, D., & Singh, R. K. (2020). Physicochemical, functional and rheological properties of grass pea (Lathyrus sativus L.) flour as influenced by particle size. *Heliyon*, *6*(11), e05471. https://doi.org/10.1016/j.heliyon.2020.e05471

Balet, S., Guelpa, A., Fox, G., & Manley, M. (2019). Rapid Visco Analyser (RVA) as a tool for measuring starch-related physiochemical properties in cereals: A review. *Food Analytical Methods*, *12*, 2344–2360. https://doi.org/10.1007/s12161-019-01581-w

Barak, S., Mudgil, D., & Khatkar, B. S. (2014). Effect of flour particle size and damaged starch on the quality of cookies. *Journal of Food Science and Technology*, *51*(7), 1342–1348. https://doi.org/10.1007/s13197-012-0627-x

Barretto, R., Buenavista, R. M., Pandiselvam, R., & Siliveru, K. (2021). Influence of milling methods on the flow properties of ivory teff flour. *Journal of Texture Studies*.

Barretto, R., Buenavista, R. M., Pandiselvam, R., & Siliveru, K. (2022). Influence of milling methods on the flow properties of ivory teff flour. *Journal of Texture Studies*, *53*(6), 820–833. https://doi.org/10.1111/jtxs.12630

Bassi, D., Kaur, K., Singh, T. P., & Kaur, J. (2021). Quality attributes and Chapatti making property of biofortified wheat as influenced by particle size. *Journal of Food Science and Technology*, *58*, 1156–1164. https://doi.org/10.1007/s13197-020-04629-6

Baye, K. (2014). *Teff: nutrient composition and health benefits* (Vol. 67). Intl Food Policy Res Inst.

Baye, K. (2018). Nutrient composition and health benefits. *The Economics of Tef, Exploring Ethiopia's Biggest Cash Crop. International Food Policy Research Institute (IFPRI), Wasington DC*, 371–396.

Baye, K., Mouquet-Rivier, C., Icard-Vernière, C., Rochette, I., & Guyot, J.-P. (2013). Influence of flour blend composition on fermentation kinetics and phytate hydrolysis of sourdough used to make injera. *Food Chemistry*, *138*(1), 430–436.

Bello, A. A., Gernah, D. I., Ariahu, C. C., & Ikya, J. K. (2020). Physico-chemical and sensory properties of complementary foods from blends of malted and non-malted sorghum, soybean and Moringa Oleifera seed flours. *Am. J. Food Sci. Technol*, *8*(1), 1–13. DOI:10.12691/ajfst-8-1-1

BeMiller, J. N. (2010). Carbohydrate analysis. In *Food analysis* (pp. 147–177). Springer.

Berski, W., Ptaszek, A., Ptaszek, P., Ziobro, R., Kowalski, G., Grzesik, M., & Achremowicz, B. (2011). Pasting and rheological properties of oat starch and its derivatives. *Carbohydrate Polymers*, *83*(2), 665–671. https://doi.org/10.1016/j.carbpol.2010.08.036

Bhuyan, D. J., & Basu, A. (2017). Phenolic compounds potential health benefits and toxicity. In *Utilisation of bioactive compounds from agricultural and food waste* (pp. 27–59). CRC Press. Doi 10.1201/9781315151540-2

Bultosa, G. (2007). Physicochemical characteristics of grain and flour in 13 tef [Eragrostis tef (Zucc.) Trotter] grain varieties. *Journal of Applied Sciences Research*, *3*(12), 2042–2051. https://www.academia.edu/download/87234090/2042-2051.pdf

Bultosa, G., Hall, A. N., & Taylor, J. R. N. (2002). Physico-chemical characterization of grain tef [Eragrostis tef (Zucc.) Trotter] starch. *Starch‐Stärke*, *54*(10), 461–468.

Bultosa, G., Hamaker, B. R., & BeMiller, J. N. (2008). An SEC− MALLS study of molecular features of water-soluble amylopectin and amylose of tef [Eragrostis tef (Zucc.) Trotter] starches. *Starch‐Stärke*, *60*(1), 8–22.

Canja, C. M., Lupu, M., & Taulea, G. (2014). The influence of kneading time on bread dough quality. *Bulletin of the Transilvania University of Brasov. Forestry, Wood Industry, Agricultural Food Engineering. Series II*, *7*(1), 79.

Carter, B. P., Galloway, M. T., Morris, C. F., Weaver, G. L., & Carter, A. H. (2015). The case for water activity as a specification for wheat tempering and flour production. *Cereal Foods World*, *60*(4), 166–170. DOI: 10.1094/CFW-60-4-0166

Central Statistical Agency (CSA). (2020). *The Federa Democratic Republic of Ethiopia Report on Area and Production of Majr Crops*. *I*.

Ch, S. (2013). Assessment of functional properties of different flours. *African Journal of Agricultural Research*, *8*(38), 4849–4852.

Chandra, S., Singh, S., & Kumari, D. (2015). Evaluation of functional properties of composite flours and sensorial attributes of composite flour biscuits. *Journal of Food Science and Technology*, *52*, 3681–3688.

Cherie, Z., Ziegler, G. R., Fekadu Gemede, H., & Zewdu Woldegiorgis, A. (2018). Optimization and modeling of teff-maize-rice based formulation by simplex lattice mixture design for the preparation of brighter and acceptable injera. *Cogent Food & Agriculture*, *4*(1), 1443381. https://doi.org/10.1080/23311932.2018.1443381

Chlopicka, J., Pasko, P., Gorinstein, S., Jedryas, A., & Zagrodzki, P. (2012). Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. *LWT-Food Science and Technology*, *46*(2), 548–555.

Codina, G.-G., Gutt, S., Gutt, G., & Mironeasa, S. (2011). Alveograph as a rheological tool to predict the quality characteristics of wheat flour. *Annals of DAAAM & Proceedings*, 1027–1029.

Collar, C., & Angioloni, A. (2014). Pseudocereals and teff in complex breadmaking matrices: Impact on lipid dynamics. *Journal of Cereal Science*, *59*(2), 145–154.

Coţovanu, I., & Mironeasa, S. (2022). An Evaluation of the Dough Rheology and Bread

Quality of Replacement Wheat Flour with Different Quinoa Particle Sizes. *Agronomy*, *12*(10), 2271. https://doi.org/10.3390/agronomy12102271

Dasa, F., & Binh, L. N. (2019). A comparative study on rheological, functional and color properties of improved Millet Varieties and Injera. *Journal of Agricultural Science and Food Research*, *10*(3), 1–8. doi: 10.35248/2593-9173.19.10.267.

Desiye, A., Abegaz, K., Negera, E., & Gobena, E. (2017). The microbiology of teff (Eragrostis tef) Enjera. *The Microbiology of Teff (Eragrostis Tef) Enjera*, *2*(2), 115–120. https://researchonline.lshtm.ac.uk/id/eprint/4654382/

do Nascimento, K. de O., Paes, S. do N. D., de Oliveira, I. R., Reis, I. P., & Augusta, I. M. (2018). Teff: suitability for different food applications and as a raw material of gluten-free, a literature review. *Journal of Food and Nutrition Research*, *6*(2), 74–81.

Dykes, L. (2019). Tannin analysis in sorghum grains. In *Sorghum* (pp. 109–120). Springer.

Eke, M. O., & Elechi, J. O. (2021). Food safety and quality evaluation of street vended meat pies sold in Lafia Metropolis, Nasarawa state, Nigeria. *Int. J. Sci. Res. in Biological Sciences*, *8*(1).

El-Alfy, T. S., Ezzat, S. M., & Sleem, A. A. (2012). Chemical and biological study of the seeds of Eragrostis tef (Zucc.) Trotter. *Natural Product Research*, *26*(7), 619–629.

Esselink, E., Van Aalst, H., Maliepaard, M., Henderson, T. M. H., Hoekstra, N. L. L., & van Duynhoven, J. (2003). Impact of industrial dough processing on structure: a rheology, nuclear magnetic resonance, and electron microscopy study. *Cereal Chemistry*, *80*(4), 419–423. https://doi.org/10.1094/CCHEM.2003.80.4.419

Fox, G., Nugusu, Y., Nida, H., Tedessa, T., McLean, G., & Jordan, D. (2020). Evaluation of variation in Ethiopian sorghum injera quality with new imaging techniques. *Cereal Chemistry*, *97*(2), 362–372. https://doi.org/10.1002/cche.10252

Gebru, Y. A., Hyun-Ii, J., Young-Soo, K., Myung-Kon, K., & Kwang-Pyo, K. (2019). Variations in amino acid and protein profiles in white versus brown teff (Eragrostis tef) seeds, and effect of extraction methods on protein yields. *Foods*, *8*(6), 202.

Ghebrehiwot, H. M., Shimelis, H. A., Kirkman, K. P., Laing, M. D., & Mabhaudhi, T. (2016). Nutritional and sensory evaluation of Injera prepared from tef and Eragrostis curvula (Schrad.) nees. Flours with sorghum blends. *Frontiers in Plant Science*, *7*, 1059. https://doi.org/10.3389/fpls.2016.01059

Gill, A., John, A., Iqbal, N., Faridi, T. A., & Noor, S. (2020). Assessment of biochemical profile among patients of Microbiological Quality Assessment of Bakery Products Available in Lahore, Pakistan: Diet Factor (Journal of Nutritional & Food Sciences), 24–29. https://doi.org/10.54393/df.v1i01.1

Girma, T., Bultosa, G., & Bussa, N. (2013a). Effect of grain Tef (Eragrostis tef (Zucc.) Trotter) flour substitutions with flaxseed on mineral content, antioxidant activity, phytic acid content and microbial quality of injera. *Science, Technology and Arts Research Journal*, *2*(3), 51–58. DOI: 10.4314/star.v2i3.98733

Girma, T., Bultosa, G., & Bussa, N. (2013b). Effect of grain tef [E ragrostis tef (Z ucc.) T rotter] flour substitution with flaxseed on quality and functionality of injera. *International Journal of Food Science & Technology*, *48*(2), 350–356.

Godebo, D. D., Dessalegn, E., & Niguse, G. (2019). Nutritional composition, microbial load and sensory properties of fenugreek (Trigonella foenum-graecum L.) flour substituted injera. *Journal of Food Processing & Technology*, *10*(7), 1–7.

Gómez, A., Ferrero, C., Calvelo, A., Añon, M. C., & Puppo, M. C. (2011). Effect of mixing time on structural and rheological properties of wheat flour dough for breadmaking. *International Journal of Food Properties*, *14*(3), 583–598.

Hopman, E., Dekking, L., Blokland, M.-L., Wuisman, M., Zuijderduin, W., Koning, F., & Schweizer, J. (2008). Tef in the diet of celiac patients in The Netherlands. *Scandinavian Journal of Gastroenterology*, *43*(3), 277–282.

Ibeabuchi, J. C., Okafor, D. C., Peter–Ikechukwu, A., Agunwa, I. M., Eluchie, C. N., Ofoedu, C. E., & Nwatu, N. P. (2017). Comparative study on the proximate composition, functional and sensory properties of three varieties of beans Phaseolus lunatus, Phaseolus vulgaris and Vigna umbellata. *International Journal of Advancement in Engineering Technology, Management and Applied Science (IJAETMAS)*, *5*(1), 1–23. https://www.academia.edu/download/53217563

Ijah, U. J. J., Auta, H. S., Aduloju, M. O., & Aransiola, S. A. (2014). Microbiological, nutritional, and sensory quality of bread produced from wheat and potato flour blends. *International Journal of Food Science*, *2014*. https://doi.org/10.1155/2014/671701

Kadan, R. S., Bryant, R. J., & Miller, J. A. (2008). Effects of milling on functional properties of rice flour. *Journal of Food Science*, *73*(4), E151–E154.

Karki, R. (2016). Study on the Preparation of Biscuit and Cake from Amaranthus and Sorghum. *Journal of Food Science and Technology Nepal*, *9*, 79–84.

Kasaye, A. T., & Jha, Y. K. (2015). *Evaluation of composite blends of fermented fenugreek and wheat flour to assess its suitability for bread and biscuit*. *4*(1), 29–35. https://doi.org/10.11648/j.ijnfs.20150401.15

Kebede, B., & Korji, D. (2017). Demonstration of improved teff varieties at selected midland districts of Guji zone, Oromia regional state, Ethiopia. *Asian Journal of Agriculture and Rural Development*, *7*(7), 131–135. https://doi.org/10.18488/journal.1005/2017.7.7/1005.7.131.135

Kelbore, T., Yilma, B., & Srinivasan, B. (2022). Value addition of Cissus quadrangularis stem powder in Ethiopian flat bread: Injera and its effect on Nutritional composition, Sensory attributes and Microbial load. *Journal of Food Science and Technology*, *59*(4), 1450–1459. https://doi.org/10.1007/s13197-021-05154-w

King, M. D. (2009). *Spoilage and preservation of food. Food quality and standards*. Eolss Publishers Co. Ltd, UK, 41.

Komlenić, D. K., Slačanac, V., & Jukić, M. (2012). Influence of acidification on dough rheological properties. In *Rheology* (pp. 266–292). IntechOpen.

Korji, D. (2018). *Demonstration of Improved Teff Varieties at Selected Midland Districts of Guji Zone*. *8*(13), 97–101.

Kumar, R., & Khatkar, B. S. (2017). Thermal, pasting and morphological properties of starch granules of wheat (Triticum aestivum L.) varieties. *Journal of Food Science and Technology*, *54*(8), 2403–2410. https://doi.org/10.1007/s13197-017-2681-x

Landillon, V., Cassan, D., Morel, M.-H., & Cuq, B. (2008). Flowability, cohesive, and granulation properties of wheat powders. *Journal of Food Engineering*, *86*(2), 178–193. https://doi.org/10.1016/j.jfoodeng.2007.09.022

Lee, C., Wang, H., & Lin, S. (2008). Effect of isomaltooligosaccharide syrup on quality characteristics of sponge cake. *Cereal Chemistry*, *85*(4), 515–521.

Lee, S.-H., Park, H.-J., Chun, H.-K., Cho, S.-Y., Cho, S.-M., & Lillehoj, H. S. (2006). Dietary phytic acid lowers the blood glucose level in diabetic KK mice. *Nutrition Research*, *26*(9), 474–479. https://doi.org/10.1016/j.nutres.2006.06.017

Liang, X., & King, J. M. (2003). Pasting and crystalline property differences of commercial and isolated rice starch with added amino acids. *Journal of Food Science*, *68*(3), 832–838. https://doi.org/10.1111/j.1365-2621.2003.tb08251.x

Lin, S., Gao, J., Jin, X., Wang, Y., Dong, Z., Ying, J., & Zhou, W. (2020). Whole-wheat flour particle size influences dough properties, bread structure and in vitro starch digestibility. *Food & Function*, *11*(4), 3610–3620.

Liu, Z., Liu, X., Fei, B., Jiang, Z., Cai, Z., & Yu, Y. (2013). The properties of pellets from mixing bamboo and rice straw. *Renewable Energy*, *55*, 1–5. https://doi.org/10.1016/j.renene.2012.12.014

Lu, Y., & Luthria, D. (2016). Influence of gelatinization on the extraction of phenolic acids from wheat fractions. *Food Chemistry*, *194*, 1138–1142.

Majzoobi, M., Pashangeh, S., Farahnaky, A., Eskandari, M. H., & Jamalian, J. (2014). Effect of particle size reduction, hydrothermal and fermentation treatments on phytic acid content and some physicochemical properties of wheat bran. *Journal of Food Science and Technology*, *51*, 2755–2761. https://doi.org/10.1007/s13197-012-0802-0

Manary, M. J., Hotz, C., Krebs, N. F., Gibson, R. S., Westcott, J. E., Broadhead, R. L., & Hambidge, K. M. (2002). Zinc homeostasis in Malawian children consuming a high-phytate, maize-based diet. *The American Journal of Clinical Nutrition*, *75*(6), 1057–1061. https://doi.org/10.1093/ajcn/75.6.1057

MATHEW, R. (2007). The RVA Handbook. *Journal of Texture Studies*, *38*(6), 755–756. https://doi.org/10.1111/j.1745-4603.2007.00124.x

McDonough, C. M., Rooney, L. W., & Serna-Saldivar, S. O. (2000). The Millets: Food Science and Technology. Handbook of Cereal Science and Technology. *CRC Press, New York, Mikulajova A, Sediva D, Certik PG, Nemeth K, Hybenova E (2017) Genotypic Variation in Nutritive and Bioactive Composition of Foxtail Millet. Cereal Res Commun*, *45*(3), 442–455.

Melese, B., Satheesh, N., Fanta, S. W., & Bishaw, Z. (2022). Nutritional, Functional, Physical, and Microstructural Properties of Ethiopian Emmer Wheat (Triticum dicoccum L.) Varieties as Affected by Growing Seasons and Grain Types (Hulled and Dehulled). *Journal of Food Quality*, *2022*.

Memon, A. A., Mahar, I., Memon, R., Soomro, S., Harnly, J., Memon, N., Bhangar, M. I., & Luthria, D. L. (2020). Impact of flour particle size on nutrient and phenolic acid composition of commercial wheat varieties. *Journal of Food Composition and Analysis*, *86*, 103358. https://doi.org/10.1016/j.jfca.2019.103358

Mezgebo, K., Belachew, T., & Satheesh, N. (2018). Optimization of red teff flour, malted soybean flour, and papaya fruit powder blending ratios for better nutritional quality and sensory acceptability of porridge. *Food Science & Nutrition*, *6*(4), 891–903.

Mihrete, Y, & Bultosa, G. (2017). The effect of blending ratio of tef [Eragrostis tef (Zucc) trotter], sorghum (sorghum bicolor (L.) moench) and faba bean (Vicia faba) and fermentation time on chemical composition of injera. *J Nutr Food Sci*, *7*(02), 1–7.

Mihrete, Yimer. (2019). The Mineral Content and Sensory Properties of Injera Made from the Faba Bean, Sorghum and Tef Flour Blend. *International Journal of Nutrition*, *4*(2), 1. DOI : 10.14302/issn.2379-7835.ijn-19-2629

Mirza Alizadeh, A., Peivasteh-roudsari, L., Tajdar-oranj, B., Beikzadeh, S., Barani-bonab, H., & Jazaeri, S. (2020). Effect of Flour Particle Size on Chemical and Rheological Properties of Wheat Flour Dough. *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*.Doi: 1021-9986/2022/2/682-694

Mishra, N., & Chandra, R. (2012). Development of functional biscuit from soy flour & rice bran. *International Journal of Agricultural and Food Science*, *2*(1), 14–20.

Mohammed, M. I. O., Mustafa, A. I., & Osman, G. A. M. (2009). Evaluation of Wheat Breads Supplemented with Teff ('Eragrostis tef (ZUCC.') Trotter) Grain Flour. *Australian Journal of Crop Science*, *3*(4), 207–212.

Munteanu, M., Voicu, G., Ștefan, E. M., Constantin, G. A., Popa, L., & Mihailov, N. (2015). Farinograph characteristics of wheat flour dough and rye flour dough. *International Symposium of ISB-INMA TEH. Agricultural and Mechanical Engineering, Bucharest, Romania, 29-31 October 2015*, 645–650.

Neela, S., & Fanta, S. W. (2020). Injera (An ethnic, traditional staple food of Ethiopia): A review on traditional practice to scientific developments. *Journal of Ethnic Foods*, *7*(1), 1–15. https://doi.org/10.1186/s42779-020-00069-x

Niazi, S., Pasha, I., Shoaib, M., Raza, H., Korma, S. A., & Abed, S. M. (n.d.). *NUTRITIONAL, PHYSIOCHEMICAL, PASTING AND THERAPEUTIC ATTRIBUTES OF COMPOSITE FLOUR*.

Nielsen, S. S. (2010). Determination of moisture content. In *Food analysis laboratory* *manual* (pp. 17–27). Springer. https://doi.org/10.1007/978-1-4419-1463-7_3

Niveditha, A. (2019). Assessment of Dough Rheological Characteristics with Mixing Time. *International Journal of Pure & Applied Bioscience*, *7*(4), 269–276. https://doi.org/10.18782/2320-7051.7601

Odedeji, J. O., & Adeleke, R. O. (2010). Pasting characteristics of wheat and sweet potato flour blends. *Pakistan Journal of Nutrition*, *9*(6), 555–557.

Olaoye, O. A., Onilude, A. A., & Oladoye, C. O. (2007). Breadfruit flour in biscuit making: effects on product quality. *African Journal of Food Science*, *1*(2), 20–23.

Onyeike, E. N., Olungwe, T., & Uwakwe, A. A. (1995). Effect of heat-treatment and defatting on the proximate composition of some Nigerian local soup thickeners. *Food Chemistry*, *53*(2), 173–175. https://doi.org/10.1016/0308-8146(95)90784-5

Pang, J., Guan, E., Yang, Y., Li, M., & Bian, K. (2021). Effects of wheat flour particle size on flour physicochemical properties and steamed bread quality. *Food Science & Nutrition*, *9*(9), 4691–4700. https://doi.org/10.1002/fsn3.2008

Parker, M. L., & Faulks, R. M. (1989). *The Contribution of Flour Components to the Structure of Injera , an Ethiopian Fermented Bread Made from Tef (Eragrostis tel)*. 10, 93–104. https://doi.org/10.1016/S0733-5210(89)80038-4

Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2013). Colour measurement and analysis in fresh and processed foods: a review. Food and Bioprocess Technology, 6, 36–60. https://doi.org/10.1007/s11947-012-0867-9

Patindol, J., Wang, Y., & Jane, J. (2005). Structure- functionality changes in starch following rough rice storage. Starch‐ Stärke, 57(5), 197–207. https://doi.org/10.1002/star.200400367

Pauwels, D. J. (2013). *Baking Properties and Resistant Starch Contents of Gluten-free Buckwheat-rice Bread Formulations*. Michigan State University. Food Science.

Piana, M. L., Oddo, L. P., Bentabol, A., Bruneau, E., Bogdanov, S., & Declerck, C. G. (2004). Sensory analysis applied to honey: state of the art. *Apidologie*, *35*(Suppl. 1), S26–S37. DOI: 10.1051/apido:2004048

Rao, B. D., Anis, M., Kalpana, K., Sunooj, K. V, Patil, J. V, & Ganesh, T. (2016). Influence of milling methods and particle size on hydration properties of sorghum flour and quality of sorghum biscuits. *LWT-Food Science and Technology*, *67*, 8–13.

Rao, M. A., Rizvi, S. S. H., Datta, A. K., & Ahmed, J. (2014). *Engineering properties of foods*. CRC press.

Rawat, S. (2015). Food Spoilage: Microorganisms and their prevention. *Asian Journal of Plant Science and Research*, *5*(4), 47–56.

Reichwald, K., & Hatzack, F. (2008). Application of a modified Haug and Lantzsch method for the rapid and accurate photometrical phytate determination in soybean, wheat, and maize meals. *Journal of Agricultural and Food Chemistry*, *56*(9), 2888–2891.

Rosell, C. M. (2011). The science of doughs and bread quality. In *Flour and breads and their fortification in health and disease prevention* (pp. 3–14). Elsevier.

Saddozai, A. A., & Samina, K. (2009). Microbiological status of bakery products available in Islamabad. *Pakistan Journal of Agricultural Research*, *22*(1/2), 93–96.

Saha, N., Goates, C., Hernandez, S., Jin, W., Westover, T., & Klinger, J. (2022). Characterization of particle size and moisture content effects on mechanical and feeding behavior of milled corn (Zea mays L.) stover. *Powder Technology*, *405*, 117535. https://doi.org/10.1016/j.powtec.2022.117535

Sapirstein, H., Wu, Y., Koksel, F., & Graf, R. (2018). A study of factors influencing the water absorption capacity of Canadian hard red winter wheat flour. *Journal of Cereal Science*, *81*, 52–59.

Saravacos, G. D., & Maroulis, Z. B. (2011). *Food process engineering operations*. CRC Press.

Serna-Saldivar, S. O., & Espinosa-Ramírez, J. (2019). Grain structure and grain chemical composition. In *Sorghum and millets* (pp. 85–129). Elsevier.

Shafie, B., Cheng, S. C., Lee, H. H., & Yiu, P. H. (2016). Characterization and classification of whole-grain rice based on rapid visco analyzer (RVA) pasting profile. *International Food Research Journal*, *23*(5).

Shimelis, E. A., Meaza, M., & Rakshit, S. (2006). *Physico-chemical properties, pasting behavior and functional characteristics of flours and starches from improved bean (Phaseolus vulgaris L.) varieties grown in East Africa*.

Shumoy, H., Lauwens, S., Gabaza, M., Vandevelde, J., Vanhaecke, F., & Raes, K. (2017). Traditional fermentation of tef injera: Impact on in vitro iron and zinc dialysability. Food Research International, 102, 93–100.

https://doi.org/10.1016/j.foodres.2017.09.092

Singh, N., Kaur, N., Katyal, M., Kaur, A., & Shevkani, K. (2017). Characteristics of starch separated from coarse and fine flour fractions obtained from hard, medium-hard, and soft Indian wheat cultivars. *Starch‐Stärke*, *69*(1–2), 1600012.

Singh, R. P., & Heldman, D. R. (2014). Fluid flow in food processing. *Taylor, SL (Ed.)*.

Skendi, A., Biliaderis, C. G., Izydorczyk, M. S., Zervou, M., & Zoumpoulakis, P. (2011). Structural variation and rheological properties of water-extractable arabinoxylans from six Greek wheat cultivars. *Food Chemistry*, *126*(2), 526–536.

Stone, H., & Sidel, J. L. (2004). Discrimination testing. *Sensory Evaluation Practices, 3rd Ed. Elsevier Academic Press, London*, 177.

Sun, X., Wu, S., Koksel, F., Xie, M., & Fang, Y. (2022). Effects of ingredient and processing conditions on the rheological properties of whole wheat flour dough during breadmaking-A review. *Food Hydrocolloids*, 108123.

Syamaladevi, R. M., Tadapaneni, R. K., Xu, J., Villa-Rojas, R., Tang, J., Carter, B., Sablani, S., & Marks, B. (2016). Water activity change at elevated temperatures and thermal resistance of Salmonella in all purpose wheat flour and peanut butter. *Food Research International*, *81*, 163–170. https://doi.org/10.1016/j.foodres.2016.01.008

Tadesse, D., Alem, T., & Wossen, T. (2016). Evaluation of Improved Varieties of Teff in West Belessa, Northwest Ethiopia. *Review of Plant Studies*, *3*(1), 1–6.

Torres-Gallo, R., Durán, R., García-Camargo, J., Morales, O., Acevedo, D., & Tirado, D. F. (2021). Pasting and Dough Rheological Properties of Ackee (Blighia sapida) Aril Flour: A Contribution to the Search for Wheat Flour Substitutes. *International Journal of Food Science*, *2021*. https://doi.org/10.1155/2021/5526912

Tsatsaragkou, K., Mandala, I., & Stoforos, N. G. (2023). Fermentation Kinetics of Gluten-Free Breads: The Effect of Carob Fraction and Water Content. *Foods*, *12*(9). https://doi.org/10.3390/foods12091809

Tsegaye, G. A. (2020). The study of functional and rheological properties of teff [Eragrostis Teff (Zucc.) Trotter] grain flour varieties. *International Journal of Food Engineering and Technology*, *4*(1), 1–8. doi: 10.11648/j.ijfet.20200401.11

Vignola, M. B., Moiraghi, M., Salvucci, E., Baroni, V., & Pérez, G. T. (2016). Whole meal and white flour from Argentine wheat genotypes: Mineral and arabinoxylan differences. *Journal of Cereal Science*, *71*, 217–223.

Urga, K., & Narasimha, H. V. (2017). Traditional sour dough bread (Difo Dabbo) making : I . Effects on phytic acid destruction. 4. Effects on phytic acid destruction. The Ethiopian Journal of Health Development (EJHD), 12(3).

Wang, H., Yang, Q., Gao, L., Gong, X., Qu, Y., & Feng, B. (2020). Functional and physicochemical properties of flours and starches from different tuber crops. *International Journal of Biological Macromolecules*, *148*, 324–332.

Weldegerima, T. M., Zeleke, T. T., Birhanu, B. S., Zaitchik, B. F., & Fetene, Z. A. (2018). Analysis of Rainfall Trends and Its Relationship with SST Signals in the Lake Tana Basin, Ethiopia. *Advances in Meteorology*, *2018*. https://doi.org/10.1155/2018/5869010

Woldemariam, F., Mohammed, A., Fikre, T., & Gebremedhin, H. (2019). *Optimization of t amaranthus-Teff-Barley flour blending ratios for better nutritional and sensory acceptability of Injer*. DOI: http://dx.doi.org/10.1080/23311932.2019.1565079

Woldemariam, F., Mohammed, A., Fikre Teferra, T., & Gebremedhin, H. (2019). Optimization of amaranths–teff–barley flour blending ratios for better nutritional and sensory acceptability of injera. *Cogent Food & Agriculture*, *5*(1), 1565079.

Woldemariam, H. W., Admassu Emire, S., Getachew Teshome, P., Toepfl, S., & Aganovic, K. (2021). Physicochemical, functional, oxidative stability and rheological properties of red pepper (Capsicum annuum L.) powder and paste. *International Journal of Food Properties*, *24*(1), 1416–1437. https://doi.org/10.1080/10942912.2021.1969945

Xiong, Y., Zhang, P., Warner, R. D., & Fang, Z. (2019). Sorghum Grain: From Genotype, Nutrition, and Phenolic Profile to Its Health Benefits and Food Applications. *Comprehensive Reviews in Food Science and Food Safety*, *18*(6), 2025–2046. https://doi.org/10.1111/1541-4337.12506

Xu, Y., Hall III, C. A., & Manthey, F. A. (2014). Effect of flaxseed flour on rheological properties of wheat flour dough and on bread characteristics. *Journal of Food Research*, *3*(6), 83. doi:10.5539/jfr.v3n6p83

Yaregal, Z., Baye, K., & Fardet, A. (2022). Compared with fresh injera, stale injera increases satiety in healthy subjects, but does not decrease the glycemic index. *Cereal Chemistry*, *99*(5), 1030–1038. https://doi.org/10.1002/cche.10556

Yaregal, Z., Baye, K., & Solomon, W. K. (2022). The influence of dough kneading time and flour particle size distribution on white bread structure, glycemic response and aspects of appetite. *Clinical Nutrition ESPEN*, *52*, 68–77. https://doi.org/10.1016/j.clnesp.2022.10.002

Yasin, U. A. (2021). Textural and eye qualities of injera prepared from quality protein maize and teff blended flours. *Int J Nutr Food Sci*, *10*(4), 78–85.

Yegrem, L., & Temesgen, D. (2019). *Evaluation of nutritional composition and sensory acceptability of tef (Eragrostis Tef (Zucc.) Trotter) complemented with lupine (Lupinus Spp.) injera*. Haramaya University. http://localhost:8080/xmlui/handle/123456789/690

Yegrem, L., Yimam, M., Kore, T., & Abebe, W. (2019). Comparative analysis of proximate and mineral composition of released Tef (Eragrostis tef (Zucc.) Trotter) varieties in Ethiopia. *Academic Research Journal of Agricultural Science and Research*, 372–379.

Yetneberk, S., de Kock, H. L., Rooney, L. W., & Taylor, J. R. N. (2004). Effects of sorghum cultivar on injera quality. *Cereal Chemistry*, *81*(3), 314–321.

Yetneberk, S., Rooney, L. W., & Taylor, J. R. N. (2005). Improving the quality of sorghum injera by decortication and compositing with tef. *Journal of the Science of Food and Agriculture*, *85*(8), 1252–1258. https://doi.org/10.1002/jsfa.2103

Yisak, H., Redi-Abshiro, M., Chandravanshi, B. S., & Yaya, E. E. (2022). Total phenolics and antioxidant capacity of the white and brown teff [Eragrostic tef (Zuccagni) trotter] varieties cultivated in different parts of Ethiopia. *Bulletin of the Chemical Society of Ethiopia*, *36*(4), 749–766. DOI: 10.4314/bcse.v36i4.3

Yousf, N., Nazir, F., Salim, R., Ahsan, H., & Sirwal, A. (2017). Water solubility index and water absorption index of extruded product from rice and carrot blend. *Journal of Pharmacognosy and Phytochemistry*, *6*(6), 2165–2168.

Yu, J., Ahmedna, M., & Goktepe, I. (2007). Peanut protein concentrate: Production and functional properties as affected by processing. *Food Chemistry*, *103*(1), 121–129. https://doi.org/10.1016/j.foodchem.2006.08.012

Zhu, F. (2018). Chemical composition and food uses of teff (Eragrostis tef). *Food Chemistry*, *239*, 402–415. https://doi.org/10.1016/j.foodchem.2017.06.101

APPENDICES

First I would like to thank you for your volunteer on sensory of injera product provided. You are provided with twelve (12) samples. Three are controls and the rest of the samples are coded and you are expected to evaluate the sensory qualities of the samples based on the hedonic scale presented here. Give score to express your degree of liking.

Please rinse your mouth between tests!

Panelist No ______ Health Status: - Normal ☐ Problem ☐

Sex: - Male ☐ Female ☐

Appendix Table 1. Sensory quality attributes evaluation form

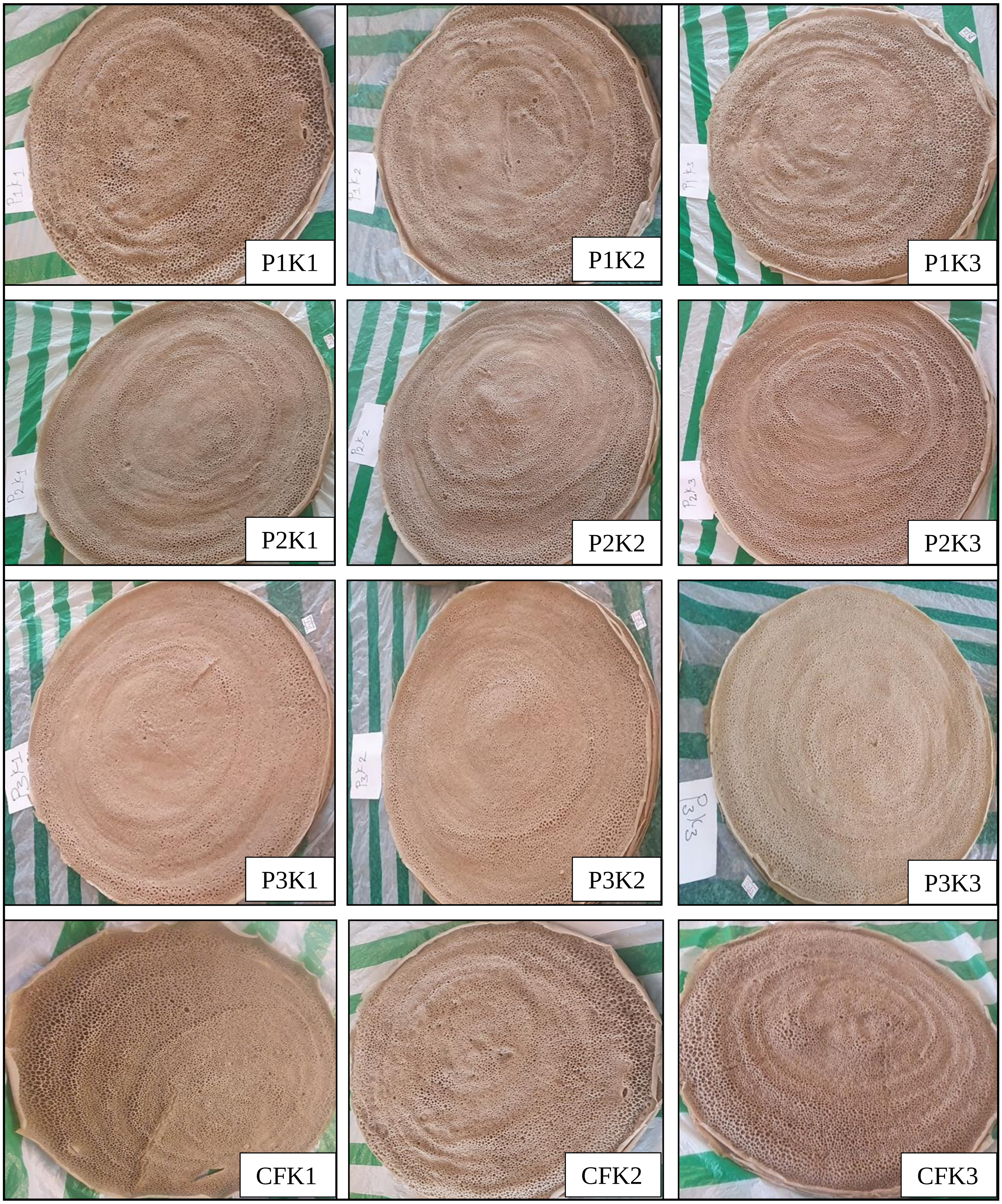
Injera Sample	Sensory Evaluation Parameters							
	Color	Taste	Eye size	Rolability	Texture	Eye Distribution	TBS	OAA
$P1K_1$								
$P1K_2$								
$P1K_3$								
$P2K_1$								
$P2K_2$								
$P2K_3$								
$P3K_1$								
$P3K_2$								
$P3K_3$								
CFK_1								
CFK_2								
CFK_3								

OAA= Over all acceptability, TBS=Top and bottom surfaces

Hedonic Scale and respective scale points/score:

Dislike very much 1, Dislike slightly 2, Neither like nor Dislike 3, like moderately 4 and like very much 5

Thank you for your time and honest evaluation! Appreciated!



Appendix Figure 1. Injera Prepared from different teff fractions and Dough kneader

Appendix Figure 2. Drying injera samples for analysis

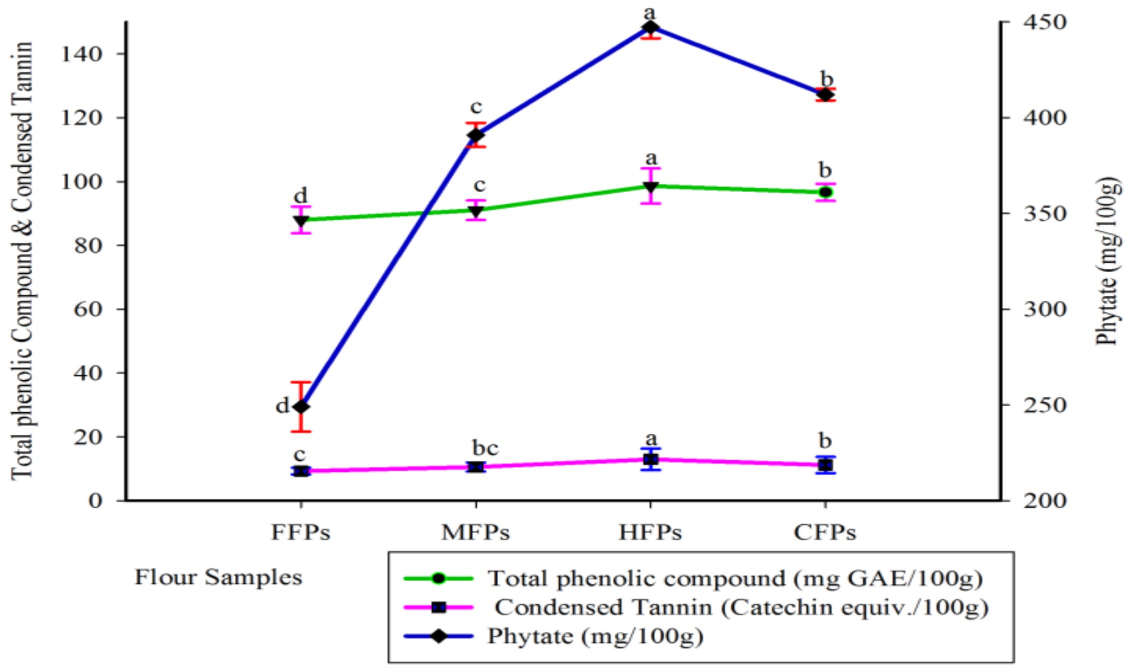
Appendix.Figure 3. Pictures of injera samples for microbial analysis

Appendix Figure 4. Sensory evaluation of injera samples

Appendix Table 2. Effect of flour particle size and dough kneading time on sensory quality of injera

Factors		Sensory attributes							
FPs	DKt	Color	Taste	Eye Size	Rollability	Texture	Eye Distribution	Top & Bottom Surface	OAA
FFPs	4 min	4.44 ± 0.81^{ab}	4.22 ± 0.82^{ab}	4.05 ± 0.80^{ab}	4.32 ± 0.85^{ab}	4.29 ± 0.81^{ab}	4.07 ± 0.93^{ab}	4.32 ± 0.76^{a}	4.29 ± 0.72^{a}
	8 min	4.59 ± 0.59^{a}	4.39 ± 0.83^{ab}	4.56 ± 0.63^{a}	4.66 ± 0.57^{a}	4.56 ± 0.59^{a}	4.59 ± 0.55^{a}	4.49 ± 0.68^{a}	4.56 ± 0.55^{a}
	12 min	4.54 ± 0.84^{ab}	4.56 ± 0.71^{a}	4.63 ± 0.66^{a}	4.66 ± 0.73^{a}	4.68 ± 0.69^{a}	4.56 ± 0.71^{a}	4.66 ± 0.57^{a}	4.66 ± 0.69^{a}
MFPs	4 min	2.61 ± 0.92^{cd}	2.83 ± 1.05^{cde}	2.68 ± 0.99^{cd}	2.95 ± 1.09^{c}	2.63 ± 0.97^{cd}	2.83 ± 1.05^{de}	3.00 ± 0.84^{c}	2.73 ± 0.71^{cd}
	8 min	2.88 ± 0.81^{cd}	2.98 ± 0.85^{cd}	2.56 ± 0.95^{cd}	3.00 ± 0.97^{c}	2.73 ± 1.05^{cd}	2.66 ± 1.13^{de}	2.76 ± 0.92^{cd}	2.78 ± 0.94^{cd}
	12 min	3.20 ± 0.93^{c}	3.15 ± 0.85^{c}	3.15 ± 0.82^{c}	3.12 ± 1.05^{c}	3.02 ± 0.72^{c}	3.02 ± 1.04^{cd}	3.15 ± 0.85^{bc}	3.10 ± 0.83^{c}
HFPs	4 min	1.93 ± 0.96^{e}	2.22 ± 0.99^{e}	1.56 ± 0.74^{e}	1.93 ± 0.88^{d}	1.85 ± 0.82^{e}	1.61 ± 0.77^{f}	1.83 ± 0.80^{e}	1.95 ± 0.80^{e}
	8 min	2.56 ± 0.95^{d}	2.51 ± 0.90^{de}	2.24 ± 0.99^{d}	2.71 ± 1.17^{c}	2.29 ± 0.96^{de}	2.34 ± 0.96^{e}	2.34 ± 0.94^{de}	2.44 ± 0.78^{de}
	12 min	3.02 ± 0.85^{cd}	2.71 ± 0.84^{cde}	2.59 ± 1.00^{cd}	2.93 ± 0.65^{c}	2.56 ± 0.84^{cd}	2.71 ± 0.87^{de}	2.71 ± 0.84^{cd}	2.83 ± 0.54^{cd}
CF	4 min	3.37 ± 0.83^{b}	3.93 ± 0.85^{b}	3.83 ± 1.12^{b}	3.93 ± 1.01^{b}	3.93 ± 0.93^{b}	3.61 ± 0.97^{bc}	3.66 ± 0.91^{b}	3.76 ± 0.77^{b}
	8 min	3.95 ± 0.92^{ab}	4.37 ± 0.77^{ab}	4.37 ± 0.86^{ab}	4.56 ± 0.63^{a}	4.49 ± 0.71^{ab}	4.49 ± 0.60^{a}	4.59 ± 0.63^{a}	4.54 ± 0.55^{a}
	12 min	4.39 ± 0.83^{ab}	4.27 ± 0.67^{ab}	4.54 ± 0.74^{a}	4.59 ± 0.55^{a}	4.37 ± 0.70^{ab}	4.59 ± 0.55^{a}	4.66 ± 0.57^{a}	4.56 ± 0.50^{a}
P-value		0.000	0.010	0.000	0.000	0.004	0.000	0.000	0.000

Values are mean ± standard deviation and the means that do not share a letter on superscripts in the same column are significantly different at ($P\leq0.05$), Where OAA= Overall Acceptability, FFPs, MFPs, HFPs and CF are flour particle sizes (180,355,500 and control flour) respectively and DKt= Dough kneading time of 4, 8 and 12 minute



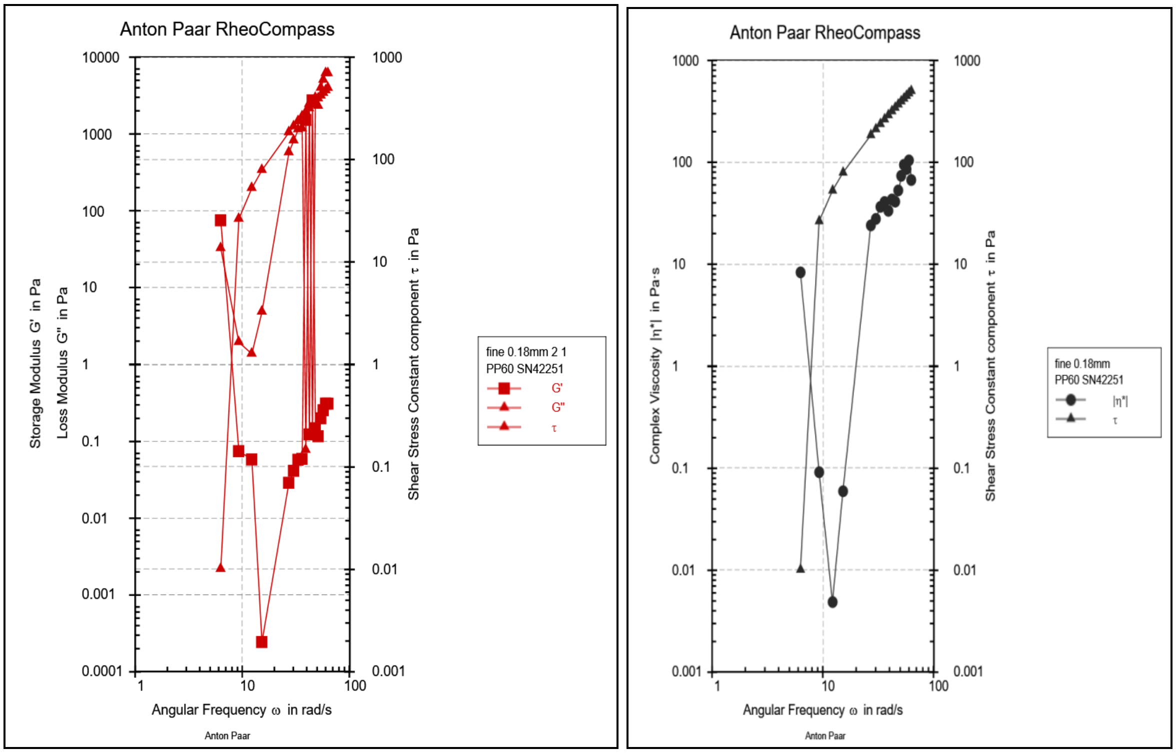
Appendix Figure 5. Effect of flour particle size on total phenolic content, phytates and condensed tannins of flour

Appendix Figure 6. Flour Particle Size of Teff Grain

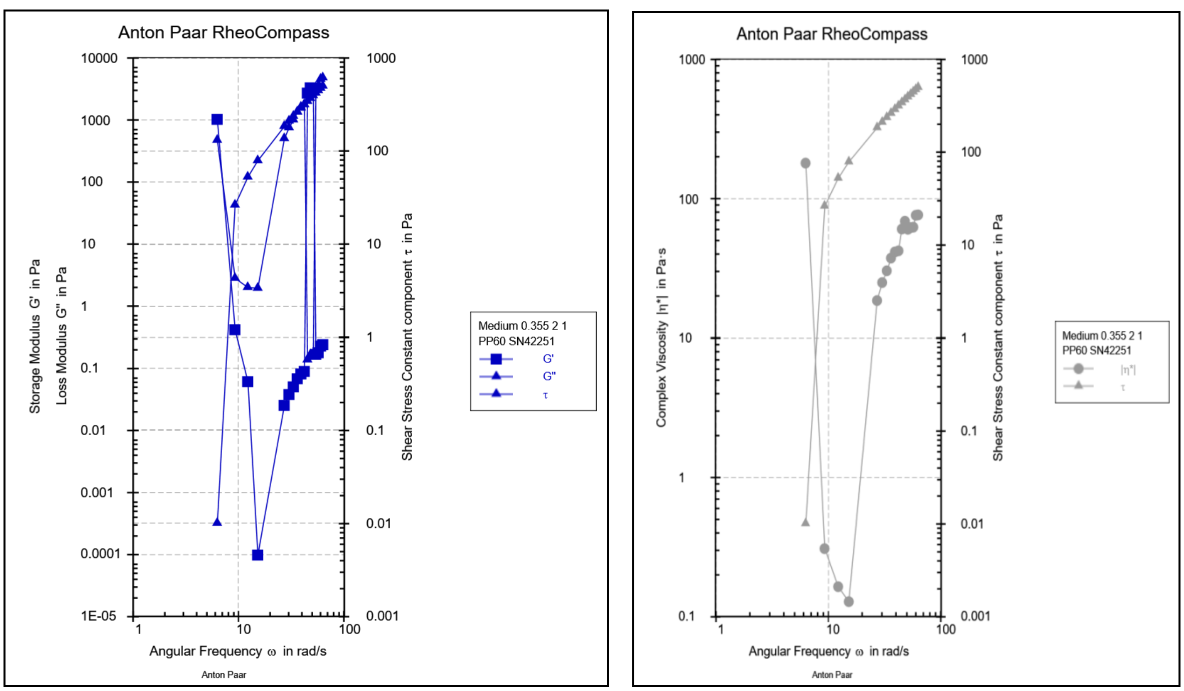
Appendix Table 3. Effect of flour particle size and dough kneading time on microbial loads of injera

Factors		Total aerobic bacterial Count in Log(CFU/g)			Yeast-Mold Count in Log(CFU/g)		
FPs	DKt	Day One	Day Three	Day Five	Day One	Day Three	Day Five
FFPs	4min	ND	3.63 ± 0.02^{cde}	4.27 ± 0.13^{abcde}	1.79 ± 0.04^{d}	3.90 ± 0.04^{ab}	4.83 ± 0.12^{abcd}
	8min	1.48 ± 0.15^{a}	3.73 ± 0.02^{bc}	4.42 ± 0.16^{abcd}	2.09 ± 0.02^{a}	4.01 ± 0.07^{ab}	4.77 ± 0.02^{bcde}
	12min	1.53 ± 0.38^{a}	3.75 ± 0.01^{b}	4.54 ± 0.07^{a}	2.09 ± 0.02^{a}	4.04 ± 0.06^{ab}	5.08 ± 0.17^{a}
MFPs	4min	ND	3.52 ± 0.03^{fg}	4.14 ± 0.18^{de}	1.81 ± 0.03^{d}	3.91 ± 0.04^{ab}	4.70 ± 0.15^{cde}
	8min	ND	3.49 ± 0.02^{g}	4.18 ± 0.06^{cde}	1.98 ± 0.04^{bc}	4.04 ± 0.07^{ab}	4.70 ± 0.14^{cde}
	12min	1.46 ± 0.06^{a}	3.61 ± 0.02^{def}	4.26 ± 0.06^{abcde}	1.98 ± 0.05^{bc}	4.07 ± 0.12^{ab}	4.79 ± 0.02^{abcd}
HFPs	4min	ND	3.52 ± 0.04^{efg}	3.97 ± 0.09^{e}	1.77 ± 0.06^{d}	3.76 ± 0.05^{b}	4.48 ± 0.07^{e}
	8min	ND	3.54 ± 0.06^{efg}	4.21 ± 0.06^{bcde}	1.82 ± 0.03^{d}	3.93 ± 0.02^{ab}	4.59 ± 0.07^{de}
	12min	1.41 ± 0.15^{a}	3.68 ± 0.04^{bcd}	4.27 ± 0.04^{abcde}	1.86 ± 0.07^{cd}	3.90 ± 0.06^{ab}	4.78 ± 0.06^{abcde}
CF	4min	ND	3.62 ± 0.01^{b}	4.42 ± 0.07^{abc}	2.06 ± 0.04^{ab}	4.14 ± 0.15^{ab}	5.02 ± 0.06^{ab}
	8min	1.42 ± 0.17^{a}	3.74 ± 0.09^{b}	4.48 ± 0.03^{ab}	2.07 ± 0.02^{ab}	3.94 ± 0.55^{ab}	4.92 ± 0.05^{abc}
	12min	1.45 ± 0.18^{a}	3.77 ± 0.02^{a}	4.49 ± 0.08^{ab}	2.01 ± 0.06^{a}	4.29 ± 0.04^{a}	5.06 ± 0.15^{ab}
P-value		0.000	0.003	0.001	0.000	0.061	0.002

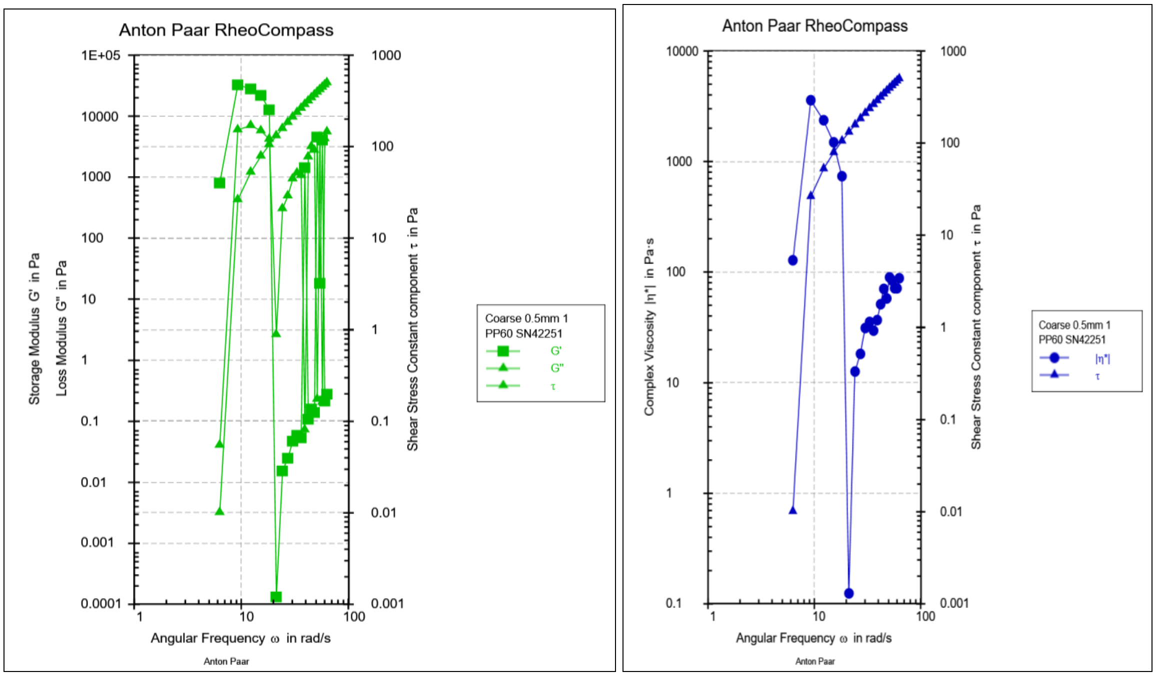
Means that do not share a letter on superscript in the same column are significantly different. Where FFPs= fine flour particle size (180µm), MFPs= medium flour particle size (355µm), HFPs=higher flour particle size (500µm) and CF= control flour



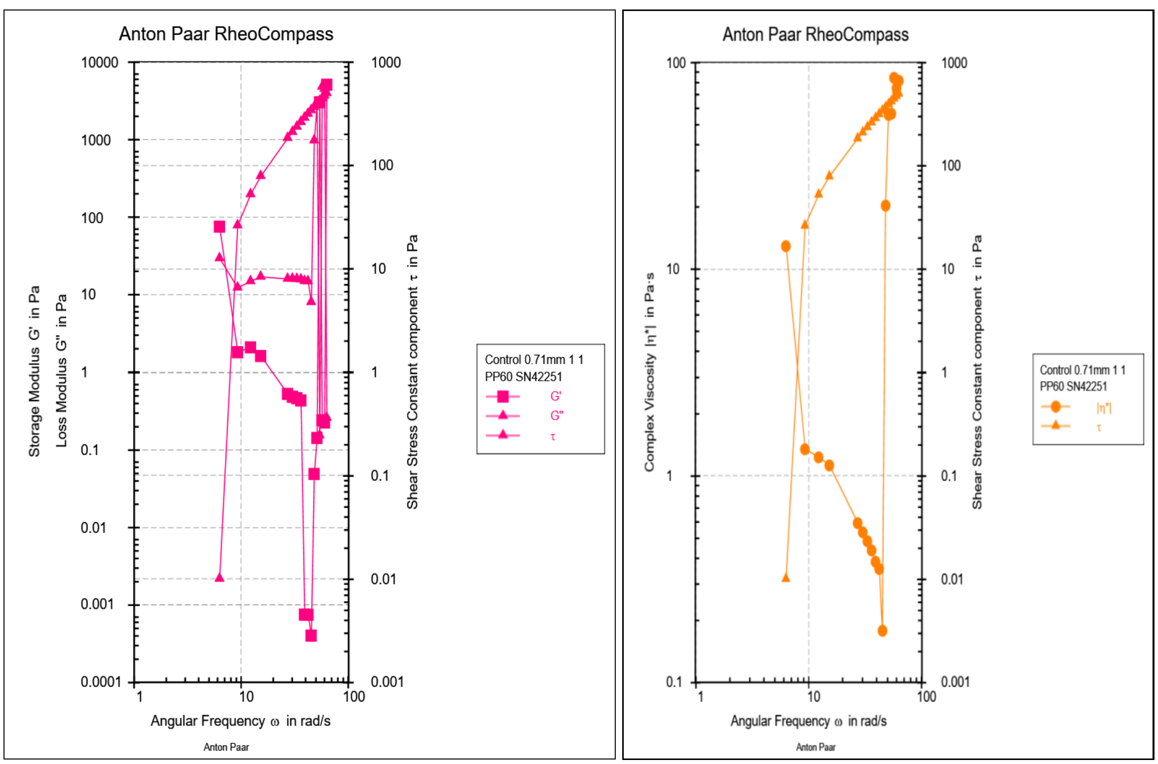
(a) Dynamic oscillatory rheology graph of fine flour particle size (180µm)



(b) Dynamic oscillatory rheology graph of medium flour particle size (355µm)



(c) Dynamic oscillatory rheology graph of coarse flour particle size (500μm)



Dynamic oscillatory rheology graph of control flour (710μm)

Appendix figure 7. Dynamic oscillatory rheology graph

Appendix Table 4. Effect of flour particle size and dough kneading time on texture of injera

Injera Samples FPs	DKt	Texture of 1st Day Storage in F/N	Texture of 2nd Day Storage in F/N	Texture of 3rd Day Storage in F/N
FFPs	4 min	15.52±2.41^{bcde}	14.04±0.13^{bcde}	12.12±0.43^{cde}
	8 min	15.25±0.31^{bcde}	12.65±1.00^{de}	11.58±2.34^{de}
	12 min	13.70±1.38^{cde}	11.44±0.99^{e}	11.12±0.33^{de}
MFPs	4 min	17.89±1.24^{ab}	17.12±1.46^{ab}	16.01±0.85^{ab}
	8 min	17.55±2.32^{abc}	16.34±3.32^{abc}	15.57±2.12^{abc}
	12 min	16.17±0.30^{ab}	13.53±0.68^{bcde}	12.09±0.85^{cde}
HFPs	4 min	19.92±1.66^{a}	18.51±2.32^{a}	16.44±3.05^{a}
	8 min	18.42±1.15^{a}	16.15±0.63^{abcd}	14.35±1.35^{bcd}
	12 min	14.10±0.42^{bcde}	13.85±0.97^{bcde}	11.92±0.97^{de}
CF	4 min	15.85±1.25^{bcd}	12.65±1.00^{de}	12.65±1.00^{bdce}
	8 min	12.33±0.41^{de}	12.30±0.59^{e}	11.57±1.34^{de}
	12 min	11.33±0.41^{e}	11.12±0.33^{e}	10.04±0.53^{e}
P-value		0.035	0.003	0.001

Means that do not share a letter on superscript in the same column are significantly different. Where FFPS= fine flour particle size (180µm), MFPs= medium flour particle size (355µm), HFPs=higher flour particle size (500µm) and CF= control flour with Kneading time of 4, 8, and 12 minute respectively

Appendix Table 5. Effect of flour particle size and dough kneading time on total phenolic content, phytates and condensed tannins of injera (mean ± SD)

Injera Samples FPs	DKt	Total Phenol (mg GAE/100g)	Condensed Tannin (mg catechin equiv./100g)	Phytates (mg/100g)
FFPs	4 min	114.86±0.11[f]	3.12±0.38[d]	218.22±7.38[de]
	8 min	115.67±0.09[f]	2.68±0.12[de]	215.08±5.02[de]
	12 min	119.30±0.13[ef]	2.26±0.05[e]	211.02±3.43[e]
MFPs	4 min	120.65±1.71[de]	4.05±0.01[bc]	252.07±10.37[b]
	8 min	121.90±0.12[cde]	3.38±0.16[cd]	238.55±2.62[c]
	12 min	123.07±0.02[cde]	3.11±0.04[d]	227.13±4.91[cd]
HFPs	4 min	126.19±1.98[abc]	5.90±0.60[a]	329.50±3.24[a]
	8 min	129.61±1.97[ab]	4.70±0.06[b]	263.49±1.69[b]
	12 min	130.59±1.82[a]	4.39±0.02[b]	258.79±0.35[b]
CF	4 min	124.35±0.13[cd]	4.12±0.37[bc]	261.39±0.17[b]
	8 min	124.21±2.74[cde]	4.05±0.32[bc]	255.15±1.54[b]
	12min	125.22±3.47[bcd]	3.37±0.38[cd]	255.93±1.40[b]
P-value		0.000	0.000	0.000

Where: - FFPs = fine flour particle size (180µm), MFPs = medium flour particle size (355µm), HFPs = higher flour particle size (500µm) and CF = Control flour and Kt= dough kneading time

Appendix Table 6. Effect of flour particle size and dough kneading time on mineral content of injera (mean ± SD)

Injera samples		Minerals (mg/100g)		
FPs	Kt	Iron (Fe)	Calcium (Ca)	Zinc (Zn)
FFPs	4 min	12.87±0.16[h]	131.70±4.19[g]	1.62±0.10[f]
	8 min	13.90±0.05[g]	135.92±2.88[fg]	1.90±0.15[de]
	12 min	14.03±0.33[g]	140.82±2.84[f]	2.03±0.08[cde]
MFPs	4 min	15.37±0.32[f]	146.80±1.31[e]	1.87±0.78[e]
	8 min	15.68±0.21[ef]	153.28±1.55[d]	2.07±0.80[bcde]
	12 min	16.20±0.23[de]	156.43±0.49[cd]	2.22±0.82[bc]
HFPs	4 min	16.62±0.06[cd]	163.40±2.05[ab]	2.20±0.50[bc]
	8 min	16.90±0.13[bc]	165.33±0.25[ab]	2.30±0.49[ab]
	12 min	17.27±0.08[ab]	165.95±0.28[a]	2.50±0.55[a]
CF	4 min	16.80±0.35[bcd]	160.22±0.28[bc]	2.12±0.80[bcd]
	8 min	17.73±0.08[a]	162.93±0.63[ab]	2.20±0.50[bc]
	12 min	17.72±0.21[a]	164.80±0.73[ab]	2.23±0.80[bc]
P-value		0.027	0.040	0.001

Where: - FFPs = fine flour particle size (180µm), MFPs = medium flour particle size (355µm), HFPs = higher flour particle size (500µm) and CF = Control flour and Kt= dough kneading time

Appendix Table 7. Effect of flour particle size and Dough kneading time on pH, TA and Batter viscosity

Dough Samples FPs	DKt	Viscosity of Batter (cP)	Batter pH	Titratable Acidity (TA)
FFPs	4 min	19.68±0.15^{a}	3.95±0.05^{bc}	1.59±0.01^{abc}
	8 min	18.99±0.12^{b}	3.93±0.03^{bc}	1.60±0.01^{ab}
	12 min	17.33±0.34^{c}	3.87±0.03^{cd}	1.63±0.01^{ab}
MFPs	4 min	14.12±0.13^{e}	4.10±0.05^{b}	1.36±0.01^{ef}
	8 min	13.25±0.09^{f}	4.02±0.08^{bc}	1.39±0.04^{def}
	12 min	12.22±0.12^{g}	3.93±0.03^{bc}	1.63±0.01^{a}
HFPs	4 min	11.21±0.04^{h}	4.33±0.03^{a}	1.27±0.01^{f}
	8 min	10.92±0.07^{h}	4.28±0.03^{a}	1.28±0.01^{f}
	12 min	10.18±0.03^{i}	4.02±0.08^{bc}	1.41±0.02^{cdef}
CF	4 min	18.66±0.09^{b}	3.98±0.03^{bc}	1.44±0.21^{bcdef}
	8 min	17.28±0.15^{c}	3.92±0.10^{cd}	1.47±0.00^{abcde}
	12 min	16.73±0.12^{d}	3.75±0.05^{d}	1.55±0.05^{abcd}
P-value		0.000	0.001	0.003

Means that do not share a letter on superscript in the same column are significantly different. Where FFPs = fine flour particle size (180µm), MFPs = medium flour particle size (355µm), HFPs =higher flour particle size (500µm) and CF = control flour

Appendix Table 8. Teff flour particle size determination obtained on sieve analysis

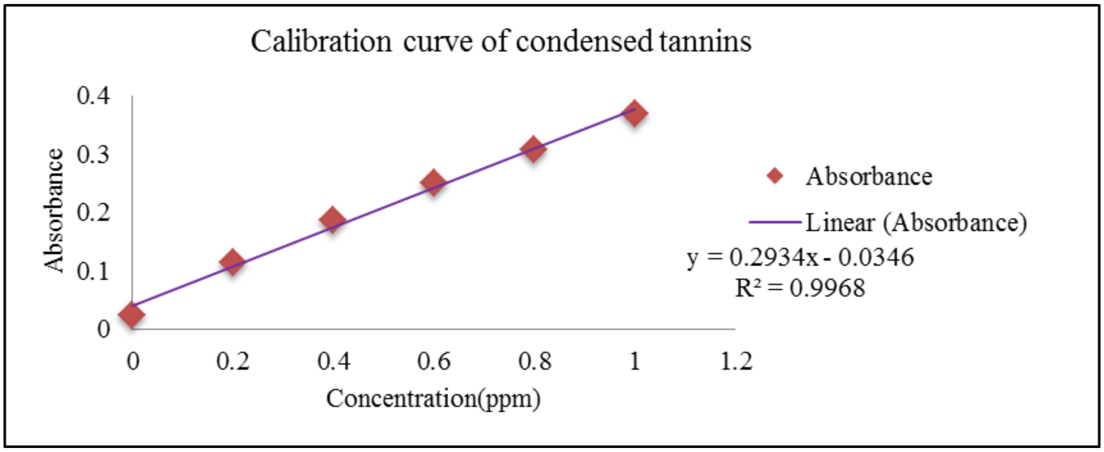
Particle Size of Flour	Size of sieve	Mass Percentage (%)
Fine Flour Particle Size (0-180μm)	180μm	30.68 ± 0.03^{c}
Control flour Particle Size (0-710μm)	710μm	99.34 ± 0.47^{a}
Medium Flour Particle Size (181-355μm)	355μm	42.42 ± 0.39^{b}
Coarse Flour Particle Size (356-500μm)	500μm	25.31 ± 0.03^{d}
P-value		0.00

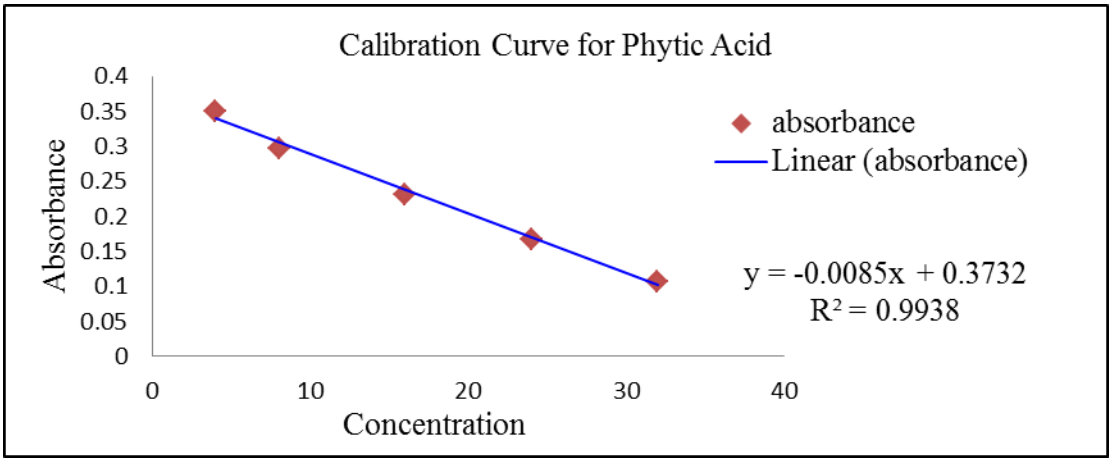
Data are expressed as the mean ± standard deviations; means that do not share a letter in the same column are significantly different

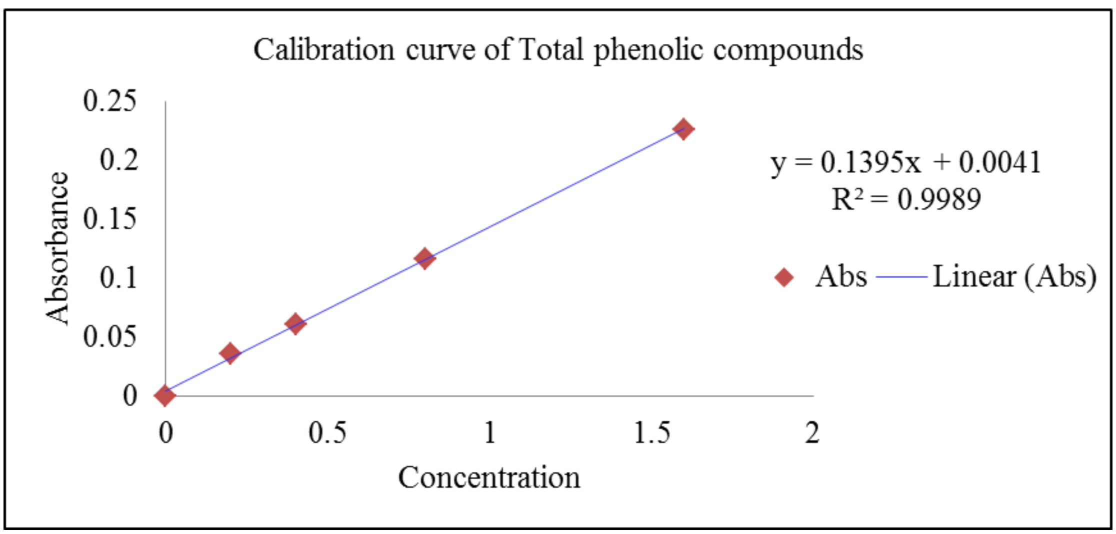
Appendix Table 9. Effect of flour particle size on mineral content of flour

Flour Samples	Minerals (mg/100g)		
	Iron (Fe)	Calcium(Ca)	Zinc(Zn)
FFPs	11.57 ± 0.68^{b}	125.83 ± 3.79^{b}	1.32 ± 0.18^{b}
MFPs	13.95 ± 0.05^{a}	133.33 ± 1.40^{b}	1.65 ± 0.05^{a}
HFPs	14.80 ± 0.13^{a}	144.47 ± 2.29^{a}	1.82 ± 0.13^{a}
CF	14.62 ± 0.28^{a}	141.98 ± 3.67^{a}	1.93 ± 0.10^{a}
P-value	0.000	0.004	0.001

Means that do not share a letter on superscript in the same column are significantly different. Where FFPs= fine flour particle size, MFPs= medium flour particle size, HFPs=higher flour particle size and CF= control flour







Appendix Figure 8. Calibration curve of Phytate, Tannin and Total phenol

Appendix Table 10. Analysis of Variance of Flour Tannin Content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Particle size in µm	3	21.07	7.02	16.79	0.001
Error	8	3.35	0.42		
Total	11	24.42			

Appendix Table 11. Analysis of Variance of Calcium Content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Particle size	3	4641.01	1547.00	425.58	0.000
Kneading time	2	254.13	127.07	34.96	0.000
Particle size*Kneading time	6	58.02	9.67	2.66	0.040
Error	24	87.24	3.64		
Total	35	5040.41			

Appendix Table 12. Analysis of Variance of Iron Content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Flour Particle Size	3	78.0019	26.0006	589.62	0.000
Dough Kneading Time	2	5.0772	2.5386	57.57	0.000
Flour Particle Size * Dough Kneading Time	6	0.7800	0.1300	2.95	0.027
Error	24	1.0583	0.0441		
Total	35	84.9174			

Appendix Table 13. Analysis of Variance of Zinc Content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Flour Particle Size	3	1.13687	0.378958	58.05	0.000
Dough Kneading Time	2	0.52792	0.263958	40.44	0.000
Flour Particle Size*Dough Kneading Time	6	0.09042	0.015069	2.31	0.037
Error	24	0.15667	0.006528		
Total	35	1.91187			

Appendix Table 14. Analysis of Variance of texture profile injera on 1st day storage

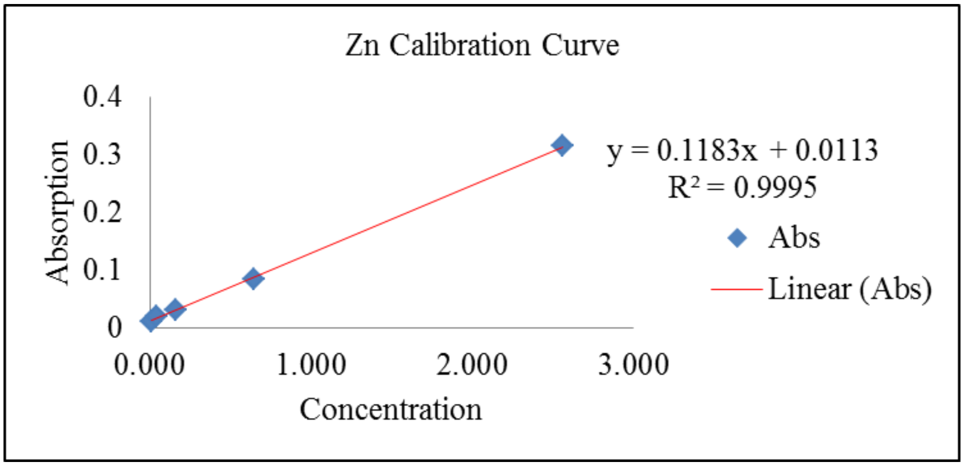
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Flour Particle Size	3	113.39	37.795	21.60	0.000
Dough Kneading time	2	70.50	35.249	20.15	0.000
Flour Particle Size*Dough Kneading time	6	28.94	4.823	2.76	0.035
Error	24	41.99	1.750		
Total	35	254.81			

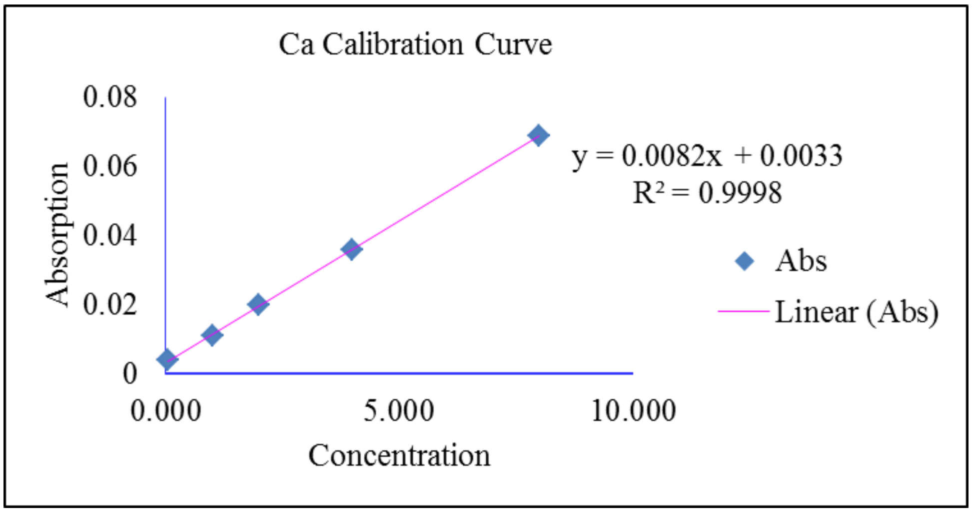
Appendix Table 15. Analysis of Variance of texture profile injera on 2nd day storage

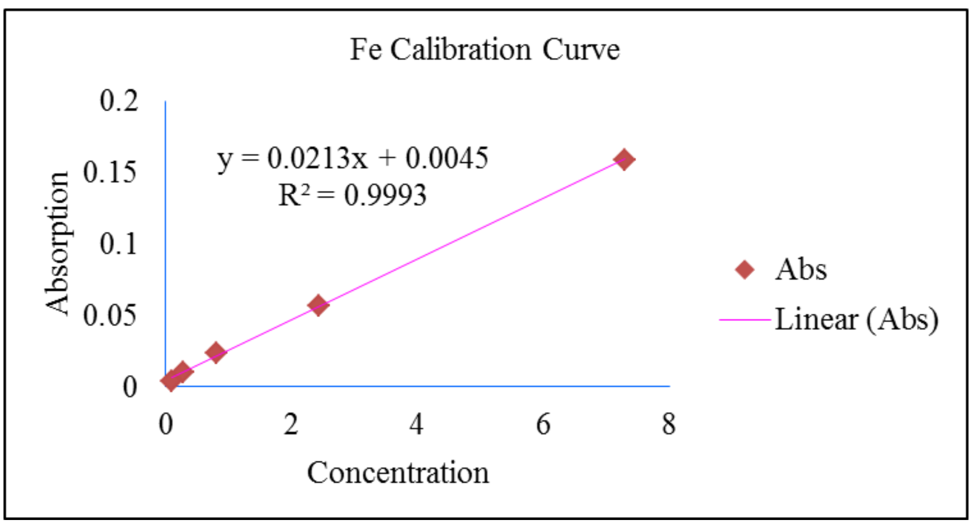
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Flour Particle Size	3	116.83	38.944	19.63	0.000
Dough Kneading time	2	13.42	6.709	3.38	0.051
Flour Particle Size*Dough Kneading time	6	54.68	9.113	4.59	0.003
Error	24	47.61	1.984		
Total	35	232.55			

Appendix Table 16. Analysis of Variance of texture profile injera on 3rd day storage

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Flour Particle Size	3	124.49	41.498	18.51	0.000
Dough Kneading time	2	46.12	23.058	10.29	0.001
Flour Particle Size*Dough Kneading time	6	107.21	17.868	7.97	0.001
Error	24	53.79	2.241		
Total	35	331.61			







Appendix Figure 9. calibration curve of minerals

Appendix Figure 10. Mechanical kneading of dough

Appendix figure 11. Dough prepared from different particle size with mechanical kneading time

Appendix Table 17. Main effect of teff flour particle size and dough kneading time on proximate composition of injera

Flour particle size	Moisture content(% db)	Crude Protein(% db)	Crude Fat(% db)	Crude Fiber(% db)	Total Ash(% db)	Carbohydrate (% db)	Gross Energy (kcal/100g)
FFPs	6.33±0.09[a]	10.75±0.89[b]	1.25±0.13[c]	2.23±0.18[d]	2.41±0.03[c]	76.93±0.99[a]	362.42±1.53[a]
MFPs	6.04±0.15[b]	11.26±0.35[a]	1.59±0.12[a]	3.21±0.12[b]	2.56±0.05[b]	75.57±0.50[bc]	361.59±1.58[b]
HFPs	5.81±0.30[c]	10.63±0.09[b]	1.27±0.05[c]	3.30±0.09[a]	2.70±0.03[a]	76.06±0.10[b]	358.21±0.63[c]
CF	6.41±0.06[a]	11.31±1.23[a]	1.45±0.19[b]	3.14±0.08[c]	2.55±0.06[b]	75.14±1.22[c]	358.85±1.60[c]
P-Value	0.000	0.001	0.000	0.000	0.000	0.000	0.000
4 min	6.15±0.16[b]	10.50±0.38[a]	1.45±0.15[a]	3.08±0.41[a]	2.57±0.11[a]	76.43±0.75[a]	361.08±2.25[a]
8 min	6.03±0.43[c]	10.77±0.24[b]	1.44±0.22[a]	2.99±0.46[b]	2.55±0.12[a]	76.21±0.66[a]	360.86±2.08[a]
12 min	6.26±0.19[a]	11.69±1.01[b]	1.29±0.15[b]	2.83±0.50[c]	2.55±0.12[a]	75.13±1.17[b]	358.86±1.83[b]
P-value	0.000	0.000	0.001	0.000	0.488	0.000	0.000

Values are mean ± SD and values in the same column with different superscript letters are significantly different from each other (P <0.05) Where: FFPs= fine flour particle size (0-180µm), MFPs= medium flour particle size (181-355µm), HFPs= higher/coarse flour particle size (356-500µm), CF= control flour

Appendix Table 18. Main effect of teff flour particle size and dough kneading time on microbial load of injera

Factors	Total aerobic bacterial Count in Log(CFU/g)			Yeast-Mold Count in Log(CFU/g)		
Flour Particle Sizes	Day One	Day Three	Day Five	Day One	Day Three	Day Five
FFPs	1.96±0.48[a]	2.74±0.12[a]	3.46±0.06[a]	2.58±0.04[a]	3.06±0.37[a]	4.00±0.10[a]
MFPs	0.89±0.27[b]	2.58±0.08[b]	3.19±0.11[b]	2.42±0.09[c]	2.98±0.08[a]	3.73±0.11[b]
HFPs	0.84±0.34[b]	2.54±0.06[b]	3.18±0.11[b]	2.32±0.06[d]	2.92±0.10[a]	3.62±0.14[b]
CF	1.88±0.42[a]	2.70±0.06[a]	3.41±0.16[a]	2.49±0.15[b]	3.01±0.04[a]	3.98±0.18[a]
P-value	0.000	0.000	0.000	0.000	0.400	0.001
Dough Kneading times						
4 min	ND	2.57±0.06[c]	3.22±0.18[a]	2.36±0.13[b]	2.94±0.13[a]	3.74±0.22[b]
8 min	1.43±0.50[b]	2.63±0.10[b]	3.32±0.16[a]	2.49±0.11[a]	2.97±0.27[a]	3.76±0.14[b]
12 min	2.74±0.27[a]	2.73±0.12[a]	3.38±0.14[b]	2.51±0.11[a]	3.07±0.16[a]	3.93±0.18[a]
P-value	0.000	0.000	0.001	0.000	0.200	0.000

Values are mean ± SD and values in the same column with different superscript letters are significantly different from each other ($P<0.05$)

Where: FFPs= fine flour particle size (0-180µm), MFPs= medium flour particle size (181-355µm), HFPs= higher/coarse flour particle size (356-500µm), CF= control flour, ND = not detected